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Throughout, change:

(1) "(Say, 1816)" to "(Say, 1817)"

(2) "(Webb, 1954a)" to "(Webb, 1954)"

(3) "Neohelix albolabris hubrichti" to "Neohelix albolabris bogani"

p. 161, right column, paragraph 3, line 8: Change "Kimura, 1982" to "Kimura, 1983"

p. 162, left column, paragraph 1, lines 5,6: Change "Woodruff & Gould 1978" to "Woodruff, 1978"

p. 163, left column, paragraph 2, line 2: Change "Babrakzai & Miller, 1975" to "Babrakzai et al., 1975"

p. 164, right column, paragraph 1, lines 11, 12: Change "Tiller, 1986" to "Tillier, 1985"

p. 167, right column, paragraph 1, lines 8-10: Omit parentheses from around dates of Tryon, Binney & Bland, and Von Martens

p. 182, left column, paragraph 2: Change "Mazyck" to "Mazýck"

p. 225, To "Triodopsis", add "Rafinesque, 1819"

To "Webbhelix", add "Emberton, new genus"

To "Xolotrema", add "Rafinesque, 1819"

p. 226, right column bottom line: Change "Grimm (1976)" to "Grimm (1975)"

p. 237, right column, paragraph 1, lines 11, 12 from bottom: Put "N. alleni alleni into italics.

p. 241, right column, lines 3,4: Change "Eberhard (1986)" to "Eberhard (1985)"

p. 247, left column, top line: Change "Gould, 1985" to "Gould, 1984"

p. 249, left column, paragraph 2, line 7: Change "chadwicki" to "chadwicki (Ferris, 1907)"

p. 249, left column, paragraph 2, line 15: Change "traversensis" to "traversensis ("Leach" Pilsbry, 1894)"

p. 257, left column, paragraph 6, line 1: Change "Studied material" to "Studied material (Syntypes)"

p. 260, right column, paragraph 5, line 1: Change "(holotype and paratypes)" to "(Syntypes)"

p. 260, right column, paragraph 5, line 18: Delete "(HOLOTYPES)"

Literature Cited: Insert the following:

Ayala, F.J., Hedgecok, D., Zumwalt, G.S. & Valentine, J.W. 1973. Genetic variation in Tridacna maxima, an ecological analog of some unsuccessful evolutionary lineages. Evolution 27: 177-191.

Bookstein, F., Chernoff, B., Elder, R., Humphries, J., Smith G. & Strauss, R. 1985. Morphometrics in Evolutionary Biology: the Geometry of Size and Shape Change, with Examples from Fishes. Academy of Natural Sciences, Special Publication 15.

Gould, S.J., 1984. Covariance sets and ordered geographic variation in Cerion from Aruba, Bonaire and Curaçao: A way of studying nonadaptation. Systematic Zoology, 33:217-237.

Hubricht, L., 1971. The land snails of South Carolina. Sterkiana, 41:41-44.

Maze, R.J. & Johnstone, C., 1986. Gastropod intermediate hosts of the meningeal worm Parelaphostrongylus tenuis in Pennsylvania: observations on their ecology. Canadian Journal of Zoology, 64: 185-188.

Miles, C.D., 1983. Land snails (Polygridae) as a source of anti-A agglutinin for typing human blood. Bulletin of the American Malacological Union, 1:97-98.

Nei, M., Tajima, F. & Tateno, Y. 1983. Accuracy of estimated phylogenetic trees from molecular data II. Gene frequency datas. Journal of Molecular Evolution, 19: 153-170.

Nichols, E.A., Chapman, V.M. & Ruddle, F.H. 1973. Polymorphism and linkage for mannosephosphate isomerase in Mus musculus. Biochemical Genetics, 8:47-53.

Literature Cited: Delete the following, which are not mentioned in the text: Carson, 1982; Dixon & Brown, 1979; Patterson & Burch, 1978; Pilsbry, 1895: Pilsbry, 1905; Pilsbry, 1946: Pilsbry, 1948; Poulick, 1957; Randles, 1900; Reeder & Rogers, 1979; Rogers et al. 1980; Shaffer, 1984; Simpson, 1944; Solem, 1972; Solem, 1975.

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THE SCORING OF POLYMORPHIC COLOUR AND PATTERN VARIATION AND ITS GENETIC BASIS IN MOLLUSCAN SHELLS

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ABSTRACT

Present modes of scoring the phenotypic and genetic variations in molluscan shell polymorphisms vary widely. Some disregard accepted conventions for distinguishing phenotypic from genetic variation, others misuse *morph* to mean any variant whether discontinuous or not. The requirements for the recognition and description of a polymorphism are discussed, and for acceptable notations for its phenotypic manifestation and genetic basis.

Key words: Shell polymorphism; discontinuous variation; morph; description; notation; symbolization.

INTRODUCTION

The methods used by Cain & Sheppard (1957), Cain, King & Sheppard (1960) and Cain, Sheppard & King (1968) for scoring colour and banding polymorphism in the snail *Cepaea* have been explicitly followed by later workers (Pettitt, 1973; Roth, 1981; Roth & Bogan, 1984) for other gastropods, both terrestrial and marine. However, as there have been considerable departures in these papers from Cain and Sheppard's methods, and as incorrectly applied methods can actually conceal the nature of the variation in a species, it is necessary to review the descriptive and notational procedures used by these and other authors, to determine which are the most suitable for the ends in view.

Continuous variation in any one character requires only a definition of that character and simple measurement or ranking. The problems discussed in the present paper are of discontinuous variation. Phenotypic variation can be either continuous or discontinuous; genetic variation, by the nature of the genes, can only be discontinuous, and if it occurs within a population (except as a rare mutation) it is necessarily a polymorphism as defined by Ford (1940, 1945). Phenotypic polymorphism is not a necessary consequence of genetic polymorphism, which may produce continuous phenotypic variation; and phenotypic polymorphism may be produced without genetic mediation as in the solitary and gregarious forms of locusts, and workers as against queens in social Hymenoptera. When mediated genetically, phenotypic polymorphism normally occurs in relation to sex, mimicry (e.g. Clarke & Sheppard 1960a, b), apostatic selection (Clarke, 1964; Clarke & O'Donald, 1964), industrial and other melanisms, and other phenomena of particular evolutionary interest. It is important, therefore, that it should be recognised, described, symbolized, and separated clearly from continuous variation.

PROTOCOL FOR A POLYMORPHISM

For a complete description and notation for a polymorphism, the following elements are required:

0.1. Descriptions of the different morphs (to use the term introduced by Huxley, 1955) either in absolute or relative terms and preferably both, e.g. for colour variation by reference to a standard colour atlas, and expressed as a difference from other forms.

0.2. Statements, with supporting evidence, of discontinuity.

0.2.1. Segregations within bred material are the best evidence (but many species cannot be bred in the laboratory).

0.2.2. Clear segregation in random samples, taken from an area small in comparison with the normal dispersal distances of the species, is also evidence that the forms concerned are morphs, not individual variants picked out of a continuum of variation. Some forms may segregate wherever they are

found, but one still needs random samples to determine their separation; museum samples are seldom random. The importance of observational evidence for discontinuous variation within populations was specially emphasized by Diver (1939).

In very large samples, or when many samples are looked at together, the full range of individual variation within morphs can be observed, and individuals apparently intermediate between otherwise distinct forms may appear. This can happen in two ways: 0.2.2.1. The segregation is clear at any one locality, but an accumulation of modifiers in a particular population, or environmental influences, may shift the expression of the alleles so that, for example, a segregation genetically of dark pink and pale pink shell colour may become in expression one of medium pink and faint pink. If all the samples are considered together, there may be a complete chain of forms connecting dark pink to pale pink. Breeding experiments will give the true explanation, but if the shift in average expression is confined to one or a few populations out of many, one may suspect the right answer merely from the samples. If a segregation appears at all, then there is a major gene difference, and the fact that it is obscured elsewhere does not abolish that finding. It does suggest that the discrete differences may be strongly affected by environmental or other genetic influences. 0.2.2.2. The expression of a morph is highly variable everywhere, and a few individuals appear intermediate between it and other morphs in any large sample. Breeding experiments are almost essential here, as in the studies of polymorphism in *Theba pisana* by Cowie (1984) and Cain (1984). Observational data can suggest the true explanation, e.g. for mantle pigmentation in *Monacha cantiana* (Cain, 1971). Here the difficulty was compounded both by the coarseness of the pigmentation, which meant that only ranking could be used, not scoring against a colour atlas, and because one form was unpigmented (except for an anal blotch) and might have been merely the extreme of variation of the pigmented form. Nevertheless the frequency distribution of the ranked forms suggested two peaks, a broad one in the pigmented class and a (necessarily) narrow one in the unpigmented. Evidence that a genetic polymorphism was indeed involved was obtained by breeding.

0.3. A notation for the morphs defined by means of 0.1 and 0.2. This may be nominal(using names) or symbolic (using letters, numbers, etc.). Names may be descriptive (e.g. *rosea, lutea, quinquefasciata*) or ascriptive (e.g. *baudonia*). Symbols can be combined more readily than names and can give a partial analysis of particular forms; for example DY00300 al in *Cepaea* has the dark yellow colour morph, the banding represented only by the middle band, and the lip of the adult shell white, without the usual pigment. Nevertheless, there are situations in which names are of use (pp. 5, 9 below).

0.4. Statements, with supporting evidence, about the genetic control of the morphs defined by 0.1 and 0.2. The evidence may be

0.4.1. segregations within random samples as in 0.2.2, merely suggesting that the polymorphism has a genetic basis;

0.4.2. observations of segregation within a sample known to be a single brood, e.g. Pilsbry (1912) on apex colour in *Liguus;* Mayer (1902) on uterine young of *Partula;* or

0.4.3. full genetic data from numerous matings or whole lineages. Since type 0.4.3 includes 0.4.2 and is of greater evidential force than 0.4.1, there is no need for 0.4.1 and 0.4.2 if 0.4.3 is obtainable. Type 0.4.1 is also open to the objection that it does not exclude the case of a polymorphism mediated purely phenotypically, a *phenomorphism* (for types of polymorphism see Cain, 1977). There is the possibility even in molluscs of such a phenomenon (e.g. in the bivalve *Corbicula,* Prezant & Chalermwat, 1984). Further, on occasion evidence of type 0.4.3 may help to clear up confusing or misleading evidence of type 0.4.1, as in the case of the snail *Theba pisana* (Cain, 1984; Cowie, 1984).

0.5. Statements of the genetic relations between the morphs, e.g. multiple allelomorphism, dominance, epistasy, linkage, complementarity, also from evidence of type 0.4.3 above. Dominance relationships are especially needed since they are not expressed in the genetic symbolization of a polymorphism (see below, p. 3).

0.6. A notation for the genetic basis as ascertained from type 0.4.3 evidence as interpreted in 0.5, conforming to normal genetic practice. As exemplified below, this notation cannot be the same as that in 0.3 since it refers not to the phenotypes but to the underlying genetic basis. Ford (1955) pointed out a similar confusion in the nomenclature then accepted for the blood groups (. . . "in the current literature it is often impossible to de-

termine whether a given symbol refers to a gene or to an antigen"). Where morphs and alleles approximately correspond, it is useful to have a corresponding similarity of reference, but not, however, an identity which could lead to confusion.

If a species can be bred easily, all these elements (0.1–0.6) can be produced, but much useful, indeed essential, work can be done directly from shell samples provided that the exact status of the specimens (see 0.2.2) is fully understood. They must form random samples of sufficient size from a restricted area (Diver, 1939, p. 114 and earlier authors referred to therein). If not, as in many highly biassed museum collections, they give no basis even for separating continuous from discontinuous variation.

SYSTEMS OF NOTATION

1. Genetic notation

The usual conventions for symbolizing genes are given in most textbooks of genetics (e.g. Avers, 1984). The principles are well set out in a proposal for a uniform nomenclature for bacterial genetics (Demerec, Adelberg, Clark & Hartman, 1968, developing a system by Demerec, 1958), which stresses the importance of distinguishing between "symbols representing the *genotype* [of a bacterial strain] and abbreviations of words which describe *phenotypic properties*". "Each locus of a given wild-type strain is designated by a three-letter, lower-case, italicized symbol", the letters being chosen to recall the phenotypic change produced by mutants, e.g. *ara* is that locus which affects the response of the cell to arabinose. A recognition that a locus so designated is composite is shown by italicized capitals, e.g. *ara A, ara B, ara D.* They recommend that all mutants should be designated by serial numbers only, since different mutants at the same locus may affect it in different ways. Their system is based on the probability that the exact sequence of nucleotides for each allele can be determined, so that the allele can be recognised as such irrespective of its effects, and the fact that the actual phenotypic effect of a given allele "may be readily altered by mutations at other loci or by changes in the environment". In the comparatively primitive state of molluscan genetics, the symbolization is more meaningful if the alleles are designated by something obviously referring to their phenotypic effects, since this is all that is known about them. Moreover, apart from dominance and epistasy and rare complementarity there seems as yet comparatively little genic interaction in visible polymorphisms of molluscs. Phenotypic effects, they recommend, should be either stated in words or abbreviated from descriptive words or phrases, the abbreviations being clearly defined the first time they appear (in a given paper). Phenotypic abbreviations should never consist of three-letter, lower-case italicized abbreviations, which are reserved for genetic loci.

Similarly in the 'Rules for nomenclature of genes, chromosome anomalies and inbred strains' put forward by an international committee for workers on mice in the 'Mouse News Letter' no. 72 (officially not a publication) it is recommended that phenotype symbols should be the same as genotype symbols "except that symbols for phenotypes should be in capitals, not italicized, and with superscripts lowered to the line". Careful provision is made for priority; the standardization of nomenclature between species to show homology is strongly recommended; and various rules are given for the symbolization of subunit structure etc., mouse genetics being a good deal more advanced than molluscan genetics.

There is now so general an agreement on the use of italicized letters for loci with italicized superscripts for alleles, in all sorts of plants and animals, that no discussion is necessary. In polymorphisms, no one allele at a locus can be singled out as wild-type, so the former use of a cross or plus sign for wild-type and letters for mutant alleles is precluded (Cain & Currey, 1963). Moreover, as there may be many alleles at a locus, the simple use of a capital letter for the dominant and a lower case of the same letter for the recessive is also precluded.

Several authors have used letters that have some meaning in relation to the descriptions of the morphs. Thus in the notation for *Cepaea* given by Cain, Sheppard & King (1968), for shell colour the locus is *C,* with alleles C^B for brown, C^{DP} for dark pink, and C^{PY} for pale yellow; *B* is used for presence or absence of banding, *S* for the presence or absence of spread bands, a form in which the banding pigment is diffused over the whole extent of the shell normally occupied by the black bands, and so on. For some morphs, the initials of old varietal names were adopted, e.g. *I* for punctate bands (var. *inter-*

rupta), since *P* was in use for degrees of pigmentation of the bands and lip. Such connection between the symbols and the morph names or descriptions is not only usual, but convenient in memorizing the symbols. Nevertheless Cain and Sheppard were careful to symbolize morphs differently from genes, by using roman upper-case letters, even if they were the same letters. Thus DP would be the phenotype of homozygous C^{DP}, but also of heterozygotes of this allele with alleles lower in the dominance hierarchy, e.g. $C^{DP}\ C^{PP}$, and $C^{DP}\ C^{PY}$. The genetic basis of a phenotype and the characteristics of that phenotype must be distinguished, as can be seen from the following considerations.

1.1. The same phenotype may have a different genetic basis in different individuals, because of dominance (just exemplified), but also because of epistasy. An unbanded white-lipped shell carries either one or two unbanding alleles B^O, but at the locus *P* it may have either white lip, P^A (albolabiate) or P^T (hyalozonate) with pigment in neither lip nor bands; since in this morph the bands are suppressed by B^O, these alleles cannot be distinguished.

1.2. Complementarity also can cause confusion. A shell with darkly pigmented bands may be, and most usually is, P^N, but rarely it may be $P^T\ P^T$ (hyalozonate, with no band pigment) combined with $O^o\ O^o$ (orange-banded, with dilute band pigment); see Cain, Sheppard & King (1968).

1.3. Without any interaction, the same phenotype may be produced by genes at different loci. An orange-banded form is produced in *Cepaea nemoralis* by O^o, but also by the allele P^L which is homologous with the *lurida* orange-banded form in *C. hortensis* (Murray, 1963; Cook & Murray, 1966; Cook, 1967).

1.4. Incomplete dominance can also produce the same phenotype by different means genetically, e.g. Wolda (1969) on *Cepaea* banding; Cowie (1984) and Cain (1984) on shell pattern in *Theba pisana*.

1.5. The same phenotype can be produced by a major gene difference in some individuals, but by an accumulation of polygenic modifiers in others. The banding form 00345, with the two upper bands missing, segregates clearly in some samples and broods of *Cepaea nemoralis,* especially on the continent of Europe, and is at a locus *T* (*trifasciata*) unlinked to that for presence or absence of bands, *B,* or to that, *U,* for reducing the five-banded form to one with only the middle band, 00300. In many British samples 00345 is connected phenotypically to 12345 by all degrees of intermediate expression of the bands, such 0:345, 10345, ::345 etc. (: marking an incomplete band) and is probably polygenically controlled. Wolda (1969), however, has evidence that some 00045 at least may be only an environmentally induced variant of 00345.

1.6. The recognition of segregants in a random sample does not always allow us to assign them to loci even when they appear to be alternatives. Thus three very common alternative states of banding in *Cepaea nemoralis* are unbanded, midbanded and five-banded, 00000, 00300, and 12345 (or some minor variant of the last). In southern England it is possible to find populations containing only one of these, or any two, or all three. It would be easy to conclude, as Diver (1932) appears to have done, that 00300 shows close linkage with the colour locus, as do 00000 and 12345. A population with only dark yellow midbandeds and dark pink unbandeds would suggest this. Yet what it really contains are the supergenes (groups of tightly linked loci) for dark yellow five-banded, $C^{DY}\ B^B$, and dark pink unbanded, $C^{DP}\ B^O$, but it is saturated with the wholly unlinked modifier U^3 which converts a five-banded into a midbanded pattern. So far from being an allele of unbanded and five-banded, midbanded is not even linked to them.

These examples, and they are not exhaustive, show the necessity of distinguishing between the morphs and their genetic bases. Furthermore, since the genetic architecture of a polymorphism (or other forms of variation) may differ in different species or even populations, a study of it and its relationships to the polymorphism is of considerable evolutionary interest.

It is recommended, therefore, that the generally accepted practice of italicized capitals for loci and italicized superscripts for alleles should be used only for genetic notation.

2. Morph notation

When Cain and Sheppard began the work on *Cepaea nemoralis,* they attempted to describe the colour and some other variation by means of the numerous varietal names listed in Taylor's *Monograph* (1914) and, like Taylor, they used von Martens's (1832) numerical system of scoring banding. The excessive bestowing of varietal names on

nemoralis shells had led to the ridiculous situation in which the same shell could be var. *castanea* because chestnut brown, *quinquefasciata* because five-banded, and *brissonia* because both brown and five-banded. That much of the variation in *Cepaea* resulted from different *combinations* of the same characters had been pointed out several times before, e.g. by Diver (1939), and by Adams (1896). Adams advocated combinations of varietal names (p. 17), e.g. *Helix nemoralis* var. *rubella-minor-albolabiata* (plus the banding formula) but remarked (p. 68) after an example with 6 names, "It is perhaps fortunate that there are certain practical limits to an infinite series." A symbolism showing the combinations was obviously much more informative than a series of ascriptive names, each unintelligible without its definition, as Diver showed (1939, p. 113). Moreover, since much of the variation could be referred to four types of character, namely shell colour, banding pattern, states of the banding (e.g. normal or punctate, fully-pigmented or unpigmented), and colour of the lip, the same simple formula, easily pronounceable and printable, could be used in tables, text, and conversation. It could be extended easily, both by addition of new types of variation as they became known (e.g. spread-banded) and by subdivision of, or addition to, existing types, e.g. dark pink, pale pink, faint pink instead of just pink. Shell colour is placed first in the formula and symbolized by roman capitals, as few as possible, e.g. Y for yellow, DY for dark yellow, FY for faint yellow, YW for yellow-white (with yellow periostracum and white calcareous layers of the shell). Superscripts are not used. This is followed by the banding formula in as much detail as required, but often reduced to unbanded 00000, midbanded 00300, and five-banded 12345, the last standing for both truly five-banded shells and all the minor variants which are probably polygenic modifiers of the same allele, B^B. 0 indicates the absence of a band. When necessary, the fusion of adjacent bands is shown by parentheses; for example a shell with formula (12)3(45) would have effectively only three bands produced by the fusion of 1 and 2 and of 4 and 5. The formulae are often abbreviated to 0, 3 and 5 as in the tables in Cain, Sheppard & King (1968). Where further detail is required, the formula can be expanded accordingly; for example Cook (1967) showed that the form 00:45 segregates from and is dominant to 00345; in such formulae a colon stands for an incomplete band (but Wolda (1969) uses a semi-colon, and the colon has also been used for a punctate band, broken up into dots). The symbol fa (fascialbate) in position 3 signifies a middle band with a white or pale stripe along one or both sides. A t instead of a number indicates a band shown only by a trace of pigment near the mouth of the adult shell. The states of the banding and lip are shown by roman letters after the band formula, for example pb for punctate bands, al for white lip (albolabiate), hz (hyalozonate) for unpigmented lip and bands. Lower-case letters are used inconsistently for states both recessive (al, hz) and dominant (pb) to the unmodified bands, and, also inconsistently, a capital S for the spread-banded form (dominant to unmodified). Occasionally an old varietal name is used, e.g. *punctata* instead of pb, also inconsistently. Murray (1963) has used the same system very effectively, with additional symbols, for the polymorphism of *Cepaea hortensis*.

For some reason that I cannot now recall, the shell colour symbols in Cain & Sheppard (1957) but not the banding formulae were printed in italics, which was certainly wrong. This may or may not have been journal usage.

This notation was originally designed to mark phenotypic segregants, and is useful, therefore, for those not yet fully described genetically as well. Thus Cain, Sheppard & King (1968) refer to the segregants PB pale brown, FP faint pink and YW yellow-white as almost certainly belonging to the colour locus *C;* their retention in roman upper-case indicates clearly that, so far, what is known of them is only their segregation.

When, as with the formula 00:45, a somewhat complex set of characters is found to constitute a morph (in this case bands 1 and 2 absent, band 3 incomplete, bands 4 and 5 normal) there is some reason for using a varietal name, and Cook (1967) gives the varieties 00345 as *listeria* and 00:45 as *donovania.* Indeed, when a very complex pattern is inherited as a whole, and is not reducible to such a series of components as is much of the variation in *Cepaea,* the varietal name is often the shortest and simplest designation. This is the case, for example, with the three morphs of wing-pattern, *dominula, medionigra* (the heterozygote) and *bimacula* of the scarlet tiger moth, *Panaxia dominula* (see Fisher & Ford, 1947, for a

coloured plate). While the excesses of varietal naming in *Cepaea* described above have no real use in notation, description or symbolization, the use of some descriptive names (e.g. *hyalozonata, roseozonata*) and some such as *donovania* can be recommended.

DEFINITIONS AND DESCRIPTIONS

3. Descriptions and figures

Taylor (1914) frequently laments the impossibility of understanding earlier varietal names published with neither figure nor description. Sometimes they may have been thought to be self-evident, being of descriptive words, e.g. *rubra, lutea,* but this is not usually good enough. Cain & Sheppard (1954) were fortunate in being able to refer to the excellent coloured figures in Taylor's *Monograph* (1914).

Descriptions should always be given of each morph at the time of its designation, to serve as definitions. Coloured figures are highly desirable but expensive, and not often satisfactory for slight but constant differences of hue, clear enough on specimens but easily obscured in printing a plate. A plate was published by Goodhart (1962) which shows several forms adequately, but the figure of a yellow with five bands fused looks more like a pink. In general, coloured plates should be printed first and scanned carefully, and a comment on their deficiencies included in the text. More usually, a reference to a standard colour atlas is all that can be given.

4. Statements of discontinuity

Cain & Sheppard (1954, p. 90) pointed out what in their samples showed clear segregation, e.g. 00000, 00300, 12345, and what was connected by frequent intermediates. (Colton (1922) did the same for varieties of the dogwhelk *Thais lapillus.*) In their purely genetical work, of course, this was obligatory, and they devoted much space (e.g. Cain, Sheppard & King, 1968) to discussion of apparent intermediates. In both breeding work and the examination of random samples it is essential to say what has been found to segregate *from what.* If form A segregates from B and from C, it does not necessarily follow that B segregates from C; compare the discussion of bred material of *Theba pisana* in Cain (1984).

USAGES BASED ON CAIN AND SHEPPARD'S

5. Pettitt (1973) on *Littorina*

Pettitt noticed the same inconvenience in scoring winkle shells as had caused Cain and Sheppard to propose their formulae for scoring *Cepaea.* He therefore proposed a system of notation for variation in *Littorina saxatilis* "based on that used for *Cepaea* as set out by Cain, Sheppard and King (1968) . . .". In his paper, however, no definitions of morphs were given, although the word morph is frequently used. In my experience, scoring large samples of this species begins easily with a number of distinct forms, but further scoring produces more and more intermediates, until many apparently obvious morphs have to be abandoned. (This was also the experience of Reimchen (1979) in *L. mariae.*) Pettitt, therefore, has at least in part confounded continuous and discontinuous variation. Breeding was not possible, since only recently have adequate techniques been produced (Atkinson & Warwick, 1983), but unfortunately Pettitt used not the morph notation but the gene notation of Cain and Sheppard, with italicized capitals and superscripts, as though the genetic basis was known. To assume, however reasonably, that the colour and banding polymorphism in *Littorina* is genetic does not warrant the use of a symbolism for loci and alleles.

Furthermore, Pettitt proposed a notation for banding with B^o for unbanded, B^1 for one-banded, B^2 for 2-banded and so on. Unbanded corresponds phenotypically, of course, to unbanded in *Cepaea,* but in this genus one-banded can be any of the formulae 10000, 02000, 00300, 00040, and 00005. Most of these are excessively rare in *Cepaea* and their genetics unknown; only 00300 is known genetically to segregate as a distinct form, and, as noted above, although it may be an alternative phenotype to 00000 and 12345, it is not an allele at their locus. In *Cepaea,* the banding morphs segregate not on the basis of the number of bands, but on the pattern. Pettitt remarks (1973, p. 532) that he had decided "to attempt a re-description of the phenotypes of *L. saxatilis* on a 'genetic' basis" (his quote marks for 'genetic') but he did not take into account the complexity of relationship between genotype and phenotype.

Pettitt does give a set of references to

various colour standards in defining his colour forms, and sufficient indications (including figures) of other variation. In scoring separately the ground-colour of the shell, banding, the colour of the bands, tessellation, interrupted lines etc. he was providing a partly analytical notation, superior to a mere naming of varieties. With no clear indication, however, of what is continuous and what is discontinuous variation, and with a tendentious interpretation of the variation by loci, the result is principally useful as a source of symbols for a properly-based classification. All his symbols should be converted to roman capitals and all superscripts demoted to the common line. His notation has then one possible advantage over Cain and Sheppard's, in that a prefixed capital (the former locus symbol) is now common to those forms that are alternatives. For ground colour of the shell, for example, his symbols for white, fawn, grey, brown and orange now become GW, GF, GG, GB and GO. A similar arrangement for colour in *Cepaea* would give CB, CDB, CDY, CPY etc., and may be useful in scoring other complex polymorphisms.

Atkinson & Warwick (1983) have used Pettitt's symbolization but rightly converted it to roman capitals, and by simplifying it they have removed most of the objectionable features. The necessity for marking their pattern symbols with an asterisk is not obvious, and although they refer to morphs, they give no statements about the continuity or discontinuity of the variation.

6. Roth (1981) on *Monadenia*

Roth's investigation of shell colour and banding variation in the helminthoglyptid snail *Monadenia fidelis* is explicitly based on random samples, with emphasis on the continuity or otherwise of the variation. Previous workers on this species had bestowed both varietal and geographical (subspecific) names. Roth states that his notation "is modelled after the systems of Cain, Sheppard, and King (1968) for *Cepaea* and Pettit [sic] (1973) for *Littorina*". In fact, his notation uses roman capitals, in agreement with Cain and Sheppard's scoring of phenotypes, but in format is of a single capital for a series of exclusive phenotypes, with superscripts for each state, thereby agreeing with Pettitt's scheme. Roth gives a table of the exact composition of his random samples, scored according to his scheme, and extensive descriptions of the different forms he recognises, with colour-atlas references, and good black-and-white photographs of some morphs.

Roth's system is incomplete in that superscripts are provided only for the ground colour of the shell, banding, and the presence or absence of a basal patch; this last appears to be based on named varieties, not on his samples. The presence of a green tinge to the basal patch is noted and symbolized, but it is not made clear whether this, like pink/not pink, is a clear-cut segregation. The banding notation proposed is of two symbols, A for the peripheral band absent, B for the shoulder band light or absent, medium, or present. Roth remarks that if the shoulder band is light or medium, "the center of the band may lack pigment; that is, the band may be rendered as two parallel lines." The illustrations suggest that the fullest banding corresponds to what in *Cepaea* would be called (12)3(45), with band 1 extending very close to the suture, and band 5, unlike in helicids, extending right to the umbilical region. To avoid prejudicing the question of homology between helminthoglyptid and helicid bands, if the band plus the basal patch are simply numbered from above downwards, the formulae for the conditions described would be (12)34 (all present); 1234 and ::34; and (if the shoulder band is absent when the peripheral band is absent, which seems to be the case in var. *semialba* Henderson) 0004. Shoulder banding is included in his statement (p. 41) that variation in his random collection is markedly discontinuous; and his descriptions indicate full pigmentation with fusion; dilute pigmentation with or without fusion; traces of banding, again with or without fusion; and total absence. In *Cepaea* the occurrence of even traces of banding indicates that the allele for bands, B^B is present; total absence of bands in our breeding stocks is given by B^O dominant to B^B, but an absence of bands could also be produced by delaying their appearance until not even traces were produced. It would be interesting to know whether the numbers of *Monadenia* without bands and with only traces in Roth's samples suggest two classes here also. Furthermore, if fusion is independent of pigmentation, except that heavily pigmented bands (dark band) are always fused, separate symbols should be used for fusion and band intensity.

Roth's carefully-based work gives us the first analytical notation for polymorphism in a helminthoglyptid.

7. Roth & Bogan (1984) on *Liguus*

This paper is a particularly welcome contribution to the literature on molluscan variation. Surely no snail has ever suffered so badly from its devotees as this unfortunate animal. As Roth and Bogan point out, a plethora of varietal, and subspecific but often not geographical names has served as the basis of remarkable theories of the species' origin and spread, which are hardly tenable if the notation for the variability is changed. They rightly remark that these epithets "tend to obscure rather than illuminate the relationship of one form to another" and "their use has canalized systematic and zoogeographic thought regarding the genus *Liguus*".

The notation they propose, of twelve categories, is of the same character as that of Roth (1981), with roman capitals as in a phenotypic notation but superscripts (for 6 categories only) as in a genetic notation. They provide a table giving their formulae for many of the varietal names already proposed, and some sketches of particular conditions (together with maps of the distributions of particular character states, and the variation of diversity in Florida). No scoring of random samples is given, however, and the problem of the use of museum material is passed over in two sentences. "The characters used here are ones in which the alternative states can be seen to segregate in randomly selected material. Most museum lots were sorted by earlier workers to conform to the standard nomenclature and cannot be used to determine whether a particular variation is discontinuous or not".

Much museum material is indeed useless for working on polymorphisms, since it is very far from being collected at random. While it is no doubt true that every one of the alternative character-states they define can be seen segregating in one or other museum lot, it is essential that full details of these lots, their scores, and why they are regarded as random should be published, both to validate the segregations proposed, and to allow other workers to consult them as standards. In the meantime, since a definite statement is made that the notation is based on segregants, the work provides a valuable basis for further studies.

One situation requires special care, namely an apparent segregation of the presence or absence of a particular character. When, as in various random samples of *Cepaea,* all the shells are clearly either unbanded or very heavily five-banded, no doubts need arise. When, however, there is considerable variation, down to near-absence, in the category *character present,* there is a serious question as to whether a continuous variation is being artificially split into an apparent polymorphism of presence/absence merely because the language does not have single-word terms for very nearly absent, nearly absent, very slightly present, etc. This problem, which is particularly acute when the variation is such as to require ranking, and direct measurement is not possible, was considered by Cain (1971) in the case of mantle pattern in the snail *Monacha cantiana.* In that case, the frequencies in the different ranks in large samples suggested a bimodality of variation probably mediated by two major alleles, plus much polygenic background variation. Here again, it is necessary to give the full data for the basis of any conclusion about segregation versus continuous variation.

OTHER USAGES

8. Dogwhelks

The complex variation in shell colour, banding and sculpture in dogwhelks (*Thais* and *Nucella*) has been studied by several authors; *Thais emarginata* is the only gastropod in which sculpture appears to be (at least in part) polymorphic (Palmer, 1984). Colton (1922) in a paper on *Thais* (or *Nucella*) *lapillus* gave definitions of 8 color morphs, with references to the Ridgway colour chart, and a clear statement that all were quite distinct. He also produced a formula for the banding by counting the maximum number of bands, and used letters, W and D to indicate a white and dark stripe. Thus a particular combination is given as:

1	2	3	4	5	6	7	8	9	10
W	D	W	W	W	D	W	D	W	DW

Narrow stripes are defined as those on a single ridge of the sculpture; wide ones include 2 or 3. In labelling both white and dark stripes, Colton has produced a very descriptive formula, but one too cumbrous to express easily the nature of the banding variation. It is as though one should describe both the bands and the interspaces of a yellow five-banded *Cepaea* as

1	2	3	4	5	6	7	8	9	10	11
Y	B	Y	B	Y	B	Y	B	Y	B	Y

instead of Y 12345.

Palmer (1984), like many other researchers on genetics, ecogenetics and evolutionary genetics, used only categories indicated by his breeding data, and has not so far produced any symbolism for alleles or loci. He gave a simple roman notation for shell colour (e.g. OR = orange, WH = white, GR-OR = grey-orange) stating explicitly which colour morphs can be recognised as discrete, and which shades of colour could not be scored reliably. In the brood in which it segregates clearly, he scored sculpture as SM (smooth) and STR (strong spirals) with continuous categories of WK (weak) and MOD (moderate) for other broods lacking a clear segregation. Such combinations of letters are more reminiscent of the words they stand for than single letters. Banding was scored only for presence or absence, corresponding genetically to two alleles at a single locus.

More recently, Palmer (personal communication) has achieved considerable genetic analysis of this very complex variation. He uses roman capitals and superscripts for his loci, and since there is now evidence that the colour in the outer part of the shell may be inherited independently of that in the inner part, e.g. on the columella, he prefixes O to the alleles and loci affecting the outer layers. Thus banding is mediated by a single autosomal locus with two alleles, banded OB^{B}, and unbanded OB^{U}, with banded dominant. Outer shell colour is symbolized as OC with variable dominance, e.g. OC^{BL} for black, OC^{OR} for orange, and is independent of OB. A further locus, for pigment intensity, is suggested with OI having no effect, OI^{R} reducing pigment intensity partially in heterozygotes, completely in homozygotes, this last resembling the usage of a superscript dash for no visible effect by Cain, Sheppard & King (1968) for some alleles in *Cepaea*. Palmer uses the $<$ symbol for 'dominant to' (e.g. $OC^{WH} > OC^{OR} > OC^{BL}$).

This seems a highly convenient symbolism, allowing for the repetition of symbols with different prefixes, so that if a locus for internal shell colour becomes necessary it can be symbolized as IC, as against OC. Since it is based on actual breeding, one might suggest that it should be printed in italics. It is worth noting that Palmer describes the banding in *Thais emarginata* as formed, not as in pulmonates by the imposition of bands of a different pigment upon various shell ground colours, but by the regularly spaced suppression of outer shell pigment.

Berry & Crothers (1974), working on large numbers of random samples of *Thais lapillus,* while giving careful descriptions of colour types, point out particular difficulties in scoring (1974, p. 125). For banding, they also find too much variation to use as yet more than presence or absence, but they illustrate patterns characteristic of particular localities.

9. *Partula*

One of the most extensive breeding programmes in land snails is that of Clarke & Murray (1969, 1971; Murray & Clarke 1966, 1976a, b) on the Pacific islands genus *Partula.* The notation of the results contrasts with that produced by Cain and Sheppard for *Cepaea,* since only varietal names have been used, even when, as in *P. taeniata,* they have dissected the variations into component loci. There are three reasons for this.

9.1. Much of the variation falls into well-defined banding patterns not easily characterized by a single descriptive word (unlike unbanded, mid-banded and five-banded in *Cepaea*). As these patterns are inherited as well-defined wholes, a simple varietal naming gives a practicable system.

9.2. The exact relationship between the component bands in different varieties is not easy to make out. While the presence or absence of a band just below the suture, another at the umbilical region, and some others in between them can be recognised with little difficulty from morph to morph, the exact number of the bands around the middle of the whorl is not easy to determine. This means that a simple numbering from above downward runs into uncertainty, and different authors might number the same lower bands differently.

9.3 Clarke & Murray rightly wished to maintain continuity with the pioneering work of Crampton (1917, 1925, 1932), who gave invaluable data on the distribution of species and of many of these distinct forms.

This is an excellent example of the virtues of varietal names, which should not be lost sight of because of the excessive use of them in *Cepaea* and *Liguus.* A black body-whorl divided by a single white band near the mid-

dle is *bisecta*, a wholly black shell *atra*, a white body-whorl with two black bands, approximately positioned so that they would frame the single white band of *bisecta*, is *frenata*. But it is not easy to say what the single broad black band of *cestata* corresponds to in the other forms. Professor J. J. Murray kindly tells me (letter of 19 July 1985) that they did consider a banding system, with 5 bands above the umbilical blot, but also discovered difficulties in homologizing the middle bands from morph to morph. As varietal names provide a good system of reference and are not tendentious since they require no homologization of bands, they allow ease and rapidity of reference while leaving the question of homology to be settled by further work. Obviously a banding homology is desirable (and Professor Murray remarks that on their system Crampton used the name *zonata* for 10305 and 1(234)5 as well as 0(234)0). The development of a numerical system and its comparison with that in helicid or helminthoglyptid snails can be considered elsewhere; here it is sufficient to point out the advantage of varietal names as labelling morphs with complex patterns, without imposing a theoretical structure of homology.

10. *Theba pisana*

Several workers on this extremely variable snail have used only broad categories; e.g. Johnson (1980, 1981) used a classification into unbanded, effectively unbanded (with the upper bands missing) and fully banded, based on a similar classification used by Cain & Sheppard (1954) when considering variation in *Cepaea* in relation to habitat. Heller (1981) used a somewhat more elaborate classification.

The first genetic analyses were presented by Cowie (1984) and Cain (1984) and necessitated a far more elaborate symbolism since good segregants are found to be characterized by highly particular banding formulae (Cain, 1984), e.g. (for the 3 upper bands) 00y, ::3, ::y, in which y indicates a yellow-buff band, not one with black pigmentation. Sacchi (1952) was the first to provide a symbolization for the extraordinarily complex patterns into which the black pigmentation of a band (when present) can be distributed, but on the basis of 4 bands, not 5, on the completely banded shell. This question is discussed by Cowie (1984).

As a result of their observations and breeding experiments, both Cain and Cowie identify a thin line almost at the upper edge of the shell whorl as band 1, so that the banding in *Theba* is basically five-banded as in *Cepaea* and many other helicid snails. Sacchi (1952 and now, personal communication) does not recognise this line as a separate band, and the four bands he recognises, numbered from above as 1, 2, 3, 4 correspond on Cain's & Cowie's interpretation to (12)345 or 02345 in *Cepaea*. Heller (1981) also recognises only 4 bands. A four-banded phenotype is extremely common in the Common Snail *Helix aspersa* which is described as four-banded by Germain (1930). But comparison of its bands with those of other helicids shows that this phenotype corresponds to 1(23)45 in *Cepaea* and other helicids. Although all present workers agree in numbering the bands from above downwards, the numbers used by different workers are therefore not homologous. Numbering is too convenient not to be used as a symbolism for repeated elements, recognisable from one shell to another. Cain's symbolism is much simpler than Sacchi's, but no doubt further breeding will produce finer discrimination of forms, as happened with 00:45 in *Cepaea* (see above). A full description will need to use something as complex as Sacchi's scheme if not more so.

Theba pisana is particularly interesting because although several morphs can be recognised, the breeding data prove that the expression of a particular allele may be somewhat variable, and occasionally shells may be produced that are indistinguishable from forms with a different genetic basis. Such a blurring will account for the fact that it is often difficult to separate all the shells of a random sample into clearcut morphs, and the variation appears to be continuous (as some of it undoubtedly is). This may be the type of variation found also in *Littorina*, and perhaps to some extent in *Liguus*. Its evolutionary significance is discussed briefly by Cain (1984). It must not be confused with the usually clearcut variation (except in minor banding varieties) found in *Cepaea* by describing it with an inappropriate symbolism.

11. *Helix aspersa*

The paper by Chevallier (1977) gives a general account of all variation in this species. Polymorphism is used simply to mean variation, but variations in size, colour and band-

ing, shape, thickness and sculpture are stated to be morphs. The word morphotype appears to be used as a synonym for morph; some morphs are thought to be subspecies. New varietal names are bestowed in the traditional style; even the commonest form is newly named var. *typica.* On the other hand, the author utilizes the banding formula as employed for *Cepaea,* and discusses the breeding experiments known to him, noting possible cases of direct influence of the environment.

Albuquerque de Matos (1985), however, distinguishes carefully between forms, varieties and morphs, recommends that the general usage of polymorphism for variation should be avoided, and gives a set of italicized symbols for the genetic variation in this species, which accord well with the suggestions in the present paper.

12. *Cochlicella acuta*

An account of the polymorphism in this species, based on breeding experiments and random sampling, is given by Lewis (1975, 1977). The same banding formulae as in *Cepaea* are shown to be applicable. In addition the states CO (continuous ostracum—opaque, usually white, shell) and DO (discontinuous ostracum—opaque shell interrupted by transparent glassy areas) in *Cochlicella* are delimited for the first time. Ground colours of the shell (amber and pale amber) have also been bred out as morphs, but are often indeterminable on particular banding and CO forms. In 1975, Lewis, like Palmer (1984) in *Thais,* gives a notation for phenotypes, but indicates the genetic basis only verbally; in his 1977 paper he gives a properly italicized notation for the supergenes, but leaves his gene nomenclature in roman (p. 426). Elsewhere in this paper (e.g., p. 449) the roman/italics convention is used fully. Lewis (1975) places an asterisk in his tables when the character is not determinable—a useful convention.

DISCUSSION

Variation has been a subject of close study ever since the publication of the *Origin of Species* (before which time it was usually thought to have no bearing on the nature of species), and, with the rise of genetics, the nature of different types of variation has been clarified considerably. One of the more remarkable differences in type of variation is that between continuous variation of the phenotype, so very common in nature and produceable both genetically and by environmental influence, and discontinuous variation. Where this latter refers only to a few very rare mutants, it is merely a necessary consequence of genetic mutation. Where, as in polymorphism as defined by Ford (1940), it involves the maintenance of high proportions of different clearcut phenotypes in their populations, it is clearly of great evolutionary interest. It may serve many different functions, as for example in apostatic polymorphism (e.g. Clarke, 1964), in polymorphic mimicry (e.g. Clarke & Sheppard 1960a, b) or as a source of genetic variation in the most familiar sort, sex. It may be genetic or not (see references and discussion in Cain, 1977).

Any work on variation, therefore, should distinguish clearly continuous and discontinuous modes, in genetically controlled variation. For discontinuous variation, Ford's definition of *polymorphism* and Huxley's of *morph,* provide a simple terminology, unfortunately often grossly misapplied. (In French, *polymorphisme* more often than not still means no more than variation, and *morph* in American (and other languages!) is usually used for anything whatever that someone wishes to distinguish.)

As some critics of an earlier draft of this paper have found difficulty with Ford's definition (1940) of polymorphism, or have felt that it has been superseded, a brief examination of it is necessary. He divides genetic variability (p. 493) into four types, "(1) disadvantageous varieties eliminated by selection and maintained at a low level by recurrent mutation of the genes controlling them; (2) variations due to the effects of genes approximately neutral as regards survival value; (3) those dependent upon genes maintained by a balance of selective agencies; and (4) advantageous varieties controlled by genes spreading through the population and displacing their allelomorphs". He points out explicitly that "The third and fourth types constitute polymorphism. Here two or more well-marked forms, capable of appearing among the offspring of a single female, occur with frequencies high enough to exclude the maintenance of the rarest of them by recurrent mutation". The expression "genetic variability" in the first sentence quoted meant in 1940 (when the exact basis of not a single polymorphism was

known) variability in the phenotype believed on good evidence to be mediated genetically, not phenotypically. While the word 'genetic' could be inserted with advantage before 'polymorphism' in the last sentence but one quoted above, the meaning of the sentence is clear, since only genetic variability is being discussed.

Those who believe they have good examples of neutral polymorphisms will presumably bring Ford's type (2) variation also under the definition of polymorphism, adding some qualification as to the frequency of the rarest form being too high to be due to immediate mutation.

Treatments subsequent to Ford's vary somewhat. For example, Mayr, Linsley & Usinger (1953, p. 96) merely equate discontinuous variation within a population with polymorphism, though the heading of the paragraph makes it clear that genetic polymorphism is meant. No mention is made of frequency. Hartl (1980, p. 77) goes straight to the genetic locus. "A POLYMORPHIC LOCUS is a locus at which the most common allele has a frequency of less than .99. Conversely a MONOMORPHIC LOCUS is one that is not polymorphic. The cut off at .99 in the definition of polymorphism is arbitrary, but it serves to focus attention on those loci with common allelic variation . . . RARE ALLELES are alleles with frequencies of less than .005 . . ." Later (p. 79) he explains "The definition of polymorphism is an attempt to focus on loci having alleles with frequencies too high to be explained solely by recurrent mutation." I prefer Ford's treatment as emphasizing discontinuity in the phenotype not mediated merely by recurrent mutation. Albuquerque de Matos (1985) has specially emphasized the distinction between phenotypic and genotypic variation, and has gone so far as to propose *populational pluralism* ("pluralismo (genetico) populacional") for the presence in a population of different alleles and one, or more generally several, loci.

In symbolizing phenotypes, apart from the general use of roman letters, there seems to be considerable variation in practice. Demerec *et al.* (1968) merely recommend words or abbreviations of them, with the requirement that the abbreviations should never be of three italicized lower case letters (as for loci). The 'Mouse News Letter' rules recommend two-, three-, or four-letter abbreviations of the name of the gene locus concerned in capitals, the name itself being "chosen so as to convey as accurately as possible the character by which the gene is usually recognised". Arabic numbers can be included but always following a letter. Cain and Sheppard used both lower-case letters and capitals, together with arabic numerals for the banding formula, which can stand by themselves when only it is in question. Their usage with regard to lower-case letters and capitals is not fully consistent. It would be simpler to elevate all letters to capitals, but a case could be made for retaining lower-case letters as symbols for qualifying words (adjectives etc.) and using capitals for substantives, or in compound symbols for using capitals for the initial letter only. In view of the complexity of variation in many molluscan shell patterns (e.g. in the prosobranch *Clithon oualaniensis,* see Grüneberg, 1976, 1978, 1979) it is thought better to make no recommendation on this point, and to wait until we know better, by experience, what flexibility is needed. Phenotype symbols can be printed on the line; there is no need to elevate their qualifying (adjectival) parts to superscripts.

In the present state of molluscan genetics, there is no molecular evidence for the homology of loci, yet it seems unnecessary to believe that the shell colour locus which produces virtually identical phenotypic effects with the same dominance relationships between the effects, and the same linkage relationships with other loci in the sibling species *Cepaea nemoralis* and *C. hortensis* is not constitutionally the same in both. Whether the red-brown and yellow-brown segregants in *Helix aspersa,* a species of a certainly very closely related genus, are genetically the same as the pink-shelled and yellow-shelled forms in *Cepaea,* but with their expression shifted towards brown, is more dubious (Cain, 1971). Nevertheless, the use by Albuquerque de Matos (1985) of *C* for the shell colour locus in *Helix aspersa,* the same symbol as used for that purpose by Cain and Sheppard in *Cepaea,* seems justified at present, in that it draws attention to the similarity of the gene expressions in these species; when we know that the loci are different (if they are) it will be time to replace or qualify the symbols. In the meanwhile, one should be very cautious in speaking of genetic homologies. Instability of the symbolization is highly undesirable, and should be avoided as far as possible. Development of it, e.g. the later distinction within the pink class P (of shell colour in *Cepaea*) of deep pink, medium pink, and pale pink, DP,

MP, PP is inevitable, but as in this example should utilize the earlier symbols and build on them. It is unfortunate that even giving a symbol may be taken to imply homology where there is none, and should therefore be explicitly disclaimed when there is no intention of asserting it. For example, a numbering of the bands from above downwards in helicid snails as 1, 2, 3, 4, 5 probably does correspond to a genuine homology, at least within the Helicinae, but its use for *Partula* cannot imply homology between this genus and the very distantly related Helicidae. Indeed, in *Partula,* since the exact nature of the banding (number of bands, relative positioning, modes of individual band variation) has not yet been worked out, names, e.g. subsutural, umbilical, are probably better than numbers. Palmer's suggestion that banding in *Thais* is by suppression of pigment, not by pigmentation of particular areas as in pulmonates points to a mere analogy between banding-patterns in some molluscs.

The examples given in the present paper are discussed both to show the interest of a comparative study of polymorphisms, and to examine the characteristics of the nomenclatures and other notations proposed so far. (Other examples of molluscan polymorphism are mentioned by Murray, 1975). From a consideration of them it is clear that

(i) discontinuous and continuous variation should be distinguished;

(ii) the nomenclature and symbolization for the morphs should be clearly separated from that for their genetic basis;

(iii) a nomenclature, using varietal names, has advantages over a symbolization when complex patterns, inherited as units are to be referred to and the homologies of their components are uncertain;

(iv) a symbolization, being analytical, has advantages over a nomenclature when it can be applied with certainty;

(v) any nomenclature or symbolization should allow easy augmentation as further information becomes available.

There can be no objection to the use of a notation such as that proposed by Roth & Bogan (1984) in place of the excessive varietal names bestowed on *Liguus fasciatus.* It does not distinguish between continuous and discontinuous variation, but neither did that of Cain and Sheppard for variation in *Cepaea,* which was therefore supplemented by explicit statements of what segregrates from what, especially since, as already described, the same phenotype may be produced both by polygenic variation and by segregation of alleles. When, as with Roth and Bogan's, a notation is proposed for variation in general, it would be preferable to print it in a different type-face; perhaps the best plan would be to print it in ordinary roman, and transfer the phenotypic notation for known morphs to bold-face. But this would involve a considerable departure from present practice, and in view of the conservatism of editors and the usual incompetence of proof-readers, is probably impracticable. At least it should be possible to restrict the word *morph* to Huxley's very useful definition, and to use the word *polymorphism* only for variation composed of morphs.

The principles given in this paper seem equally applicable to the scoring of phenotypic polymorphisms in other organisms besides molluscs.

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THE INCIDENCE AND VARIETY OF *LEHMANNIA VALENTIANA* CONJOINED TWINS: RELATED BREEDING EXPERIMENTS (GASTROPODA, PULMONATA)

Jeanine Mason[1] & Jonathan Copeland[2]

ABSTRACT

The major objective of this study was to record the incidence and types of terata occurring in *Lehmannia valentiana,* a terrestrial mollusk, and to determine if the occurrence was inheritable. A series of breeding experiments was done comparing various groups of *L. valentiana,* which seems to be a hybrid, and its proposed parents *Limax maximus* and *Deroceras reticulatum.*

L. valentiana produces conjoined twins naturally, which are frequently viable, at a higher rate than previously recorded in terrestrial mollusks. When they are raised to maturity and mated, other conjoined twins will be included among their offspring. Self-fertilizing (isolated from birth) *L. valentiana* will sometimes produce offspring, including conjoined twins.

The related limacid species, *Limax maximus* and *Deroceras reticulatum,* produced no conjoined twins during the study period.

Conjoined twins can be tentatively identified on the basis of two close zygotes (doublets) in one egg capsule immediately after oviposition or even in the capsules contained in the laying animal's oviduct.

The number of doublets and conjoined twins in a clutch of eggs can be increased by mating animals that were themselves close doublets. The occurrence of doublets can be reduced by mating paired singlets (animals originating as one zygote in a capsule) and/or maintaining a colony of animals which all originated as singlets.

Key words: *Lehmannia valentiana; Limax maximus; Deroceras reticulatum;* conjoined twins; terata; malformations; self-fertilization; hybridization.

INTRODUCTION

Conjoined twinning in the Mollusca, as in most animals, has been uncommon. Since no references to twins or "double monstrosities" in Mollusca had been cited, Newman (1923) initially concluded that twinning did not occur due to the characteristic determinate cleavage of the molluscan zygote.

Refutation followed with reports of twinning in *Serpuloides vermicularis* (a sessile tubicolous mollusc) (Hall 1925). Crabb & Crabb (1927), however, questioned whether the occurrence of more than one embryo in a single egg capsule of some pulmonates was *true* twinning. Newman (1923) said *true* twins must arise from a single cell.

In a later work, Crabb (1931) found conjoined twin embryos in several fresh-water snails. He concluded, after extensive study and some unsuccessful experimentation, that the conjoined twins arose as separate ova that fused before cleavage or during cleavage up to the early blastula stages. He claimed that the occurrence of two or more ova per capsule was not hereditary.

Bigus (1981) found an average incidence of 0.02% conjoined twins in all eggs collected from the pulmonate *Physa acuta.* When the egg capsules contained more than one ovum, the average rate was 2.78%. Concurring with Crabb (1931), Bigus suggested that conjoined twinning only occurred in egg capsules containing more than one ovum and that the trait was not inheritable.

Experimentally, molluscan separate and/or conjoined twins have been produced by compression of a zygote (Guerrier, 1970). George (1958) obtained three conjoined twins by chemically treating 200 egg capsules containing two or more zygotes and then centrifuging them. Whether experimentally induced or occurring naturally, none of the conjoined twins previously studied have reached the hatching stage.

While we were studying the progeny of 30 *Lehmannia valentiana* (Férussac) that we had obtained from the egg capsules of

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Limax maximus Linnaeus [*L. valentiana* may be a hybrid obtained by crossing *L. maximus* and *Deroceras reticulatum* Müller (Mason, 1985)], we observed a healthy, two-headed, one-tailed animal among the other newly-hatched slugs. Microscopic examination of unhatched capsules revealed other abnormal animals.

By maintaining a laboratory-raised population of *L. valentiana* for several generations, we were able to obtain, rear, and mate a considerable number of viable conjoined twins and other anomalies, such as fused tentacles, supernumerary eyes, etc.

The data collected included the rates of conjoined twinning in *L. valentiana* and its putative parents, *L. maximus* and *D. reticulatum,* and the occurrence rate of two or more ova per egg capsule and whether this rate could be increased or decreased by selective breeding. We also catalogued the kinds of conjoined twins and other anomalies.

MATERIAL AND METHODS

D. reticulatum animals were collected locally in Wisconsin. *L. maximus* animals were laboratory-raised from an original group of animals from New Jersey. Thirty founder *L. valentiana* were obtained from eggs collected from a *L. maximus* colony. We concluded that *L. valentiana,* identified by L. F. Chichester (personal communication, 1981), must be a hybrid. Individuals from a natural population of *L. valentiana* were collected in Lexington, Kentucky, by David Prior.

All animals were maintained using methods similar to Reingold & Gelperin (1980). In addition to the lab chow, they were given fresh lettuce and Gerber baby food green beans. *L. valentiana* fed only lab chow did not produce fertile eggs, but animals fed any two of these three foods did so. Tegosept M, a mold inhibitor, used by Reingold & Gelperin (1980), was not added to the food.

All animals were maintained with an artificial photoperiod consisting of long days (LD 16:8) in an attempt to maximize reproduction (Sokolove & McCrone, 1978).

Eggs were collected from all groups at least once weekly during periods of reproduction. The eggs were washed in a strainer under tap water and then placed in labeled petri dishes lined with filter paper cut to fit in the dish. The eggs were placed within a large central hole cut in the filter paper. This procedure facilitated viewing the embryos under the microscope. The paper was kept uniformly moist throughout the embryonic growth period.

Newly hatched slugs were transferred to 12 cm diameter by 7 cm deep plastic dishes lined with filter paper. Micropore tape over holes in the cover provided ventilation while preventing escape. Abnormal and/or conjoined twins were separated from normal animals. The animals were transferred to larger cages as they matured.

Percentages of eggs with conjoined twins and other defects in embryos which completed embryonic development were determined for the laboratory populations of *L. maximus* and *D. reticulatum,* the original group and three further generations of *L. valentiana* and the field-collected *L. valentiana* from Kentucky. The percentages of conjoined twins and other defective embryos were also determined for two *L. valentiana* we obtained by crossing *L. maximus* and *D. reticulatum,* and for a self-fertilizing animal. Three *L. valentiana* were individually isolated to determine if *L. valentiana* could self-fertilize and produce conjoined twins. Parthenogenesis, while unknown in pulmonates, is unlikely but not excluded (McCracken & Selander, 1980). Determination of self-fertilization and the production of conjoined twins could demonstrate the ability of a single hybrid to found a population containing the trait. These data are presented in Table 1.

Several abnormally developed groups of *L. valentiana* were maintained, as explained below, and eggs from these animals were examined to determine the percentage of conjoined twins and other defects in the embryos which completed embryonic development (Table 2). We wanted to determine if animals with specific abnormalities such as "fused tentacles" or "two-head, one-tail" were fertile and whether they would produce progeny with similar abnormalities. All matched anomalies were kept together but isolated from other categories of animals from the time they hatched.

The percentage of egg capsules with multiple embryos was determined for the following groups: *D. reticulatum, L. maximus,* and the *L. valentiana* mixed colony, Kentucky colony, singlet colony, doublet colony, paired close doublets, paired singlets and one self-fertilizing animal (Table 3).

No selection was utilized in the mixed col-

onies. In the singlet colony, animals were obtained by examination of egg capsules after oviposition and selection of capsules that contained one ovum. These animals were raised as a colony but isolated from other categories of animals.

Doublet colony animals were obtained by selecting egg capsules which contained two ova. These animals were then raised together in colonies, i.e. all animals originated as doublets.

Four pairs of paired close doublets were obtained by microscopic examination of capsules after oviposition. Capsules containing two ova close enough together that it was impossible to measure the distance separating them at × 100 magnification were designated as "close doublets." Just prior to hatching, the capsules were opened manually with forceps to ensure that the animals would not hatch by themselves and mix with other hatching animals. These paired close doublets were then raised to sexual maturity completely isolated from other categories of animals.

Three pairs of paired singlet animals were obtained by taking animals which had developed singly in an egg capsule and pairing them, isolated from other animal categories, throughout their lives.

The self-fertilizing animal was isolated from the time of hatching.

RESULTS

Morphology

The many variations of conjoined twinning and other defects in *L. valentiana* are shown in Fig. 1. All animals shown with the characteristic mantle line of *L. valentiana* reached the hatching stage (approx. 20–22 days after oviposition). Numbers 48 and 49 are only two examples of forms of conjoining where the animals did not reach full maturity during a normal development period. Abnormal embryos arrested in development (Fig. 2) frequently remained responsive to tactile stimuli, such as tapping the capsule with a forceps, for a month after the hatching due date. Many would eventually die, but in some, growth continued at a slow rate until the animal reached hatch status. Death was determined by tissue opaqueness and/or cell disintegration.

We found that tentacle and eye abnormalities (Fig. 1) were often associated with the twinning process. Opening capsules manually with forceps revealed that many abnormalities occurred in an animal that had developed as one of a doublet (two ova per egg capsule). To determine if there was consistency in this observation, we opened 150 egg capsules containing doublets, or a singlet and an amorphous mass, or a developed doublet with an arrested development sibling. In 17 cases, we found that, whereas one of the doublet pair might be normal, the other had various deformities, e.g. supernumerary or missing eyes; missing, fused or additional tentacles; mantle deformities and/or mouth deformities. Abnormal slugs found with amorphous tissue (numbers 13 and 14) in which eyes or other body parts were identifiable provided a clue that many of these anomalies might also be forms of conjoined twinning. Newman (1923) noted that additional appendages or organs could indicate an initial case of conjoined twinning.

Tentacle and/or eye abnormalities (including the absence of eyes) were numerous both in combination with other morphological doubling and separately (numbers 1 to 6, 44). Although extra eyes under the mantle occurred occasionally (number 4), they were not always in the position denoted by the arrow and did not always seem to be located within the ocular tentacle. Seven was the maximum number of eyes on one tentacle (number 8). Number 22 had at least six eyes on the third ocular tentacle. Therefore, the maximum number of eyes observed per animal was eight (this could be higher because some eyes seem to be fused). Arrows at numbers 20, 24, and 33 illustrate what appeared to be cyclopia, or the fusion of one or more eyes.

Fused tentacle slugs [Fig. 1 (No. 3), Fig. 3] were initially identified in offspring of the original colony. The defect was frequently found in doublet capsules in which one embryo had ceased development.

In most occurrences, the conjoined twins or parts thereof are aligned anterior to anterior. Numbers 27–30 illustrate animals where there is some deviation from this alignment. Number 27 was the only animal observed that was fused with the heads orthogonal to the tails. The animal lived several months, functioned well and moved with no apparent difficulty (Fig. 7).

The posterior vestige of an incorporated

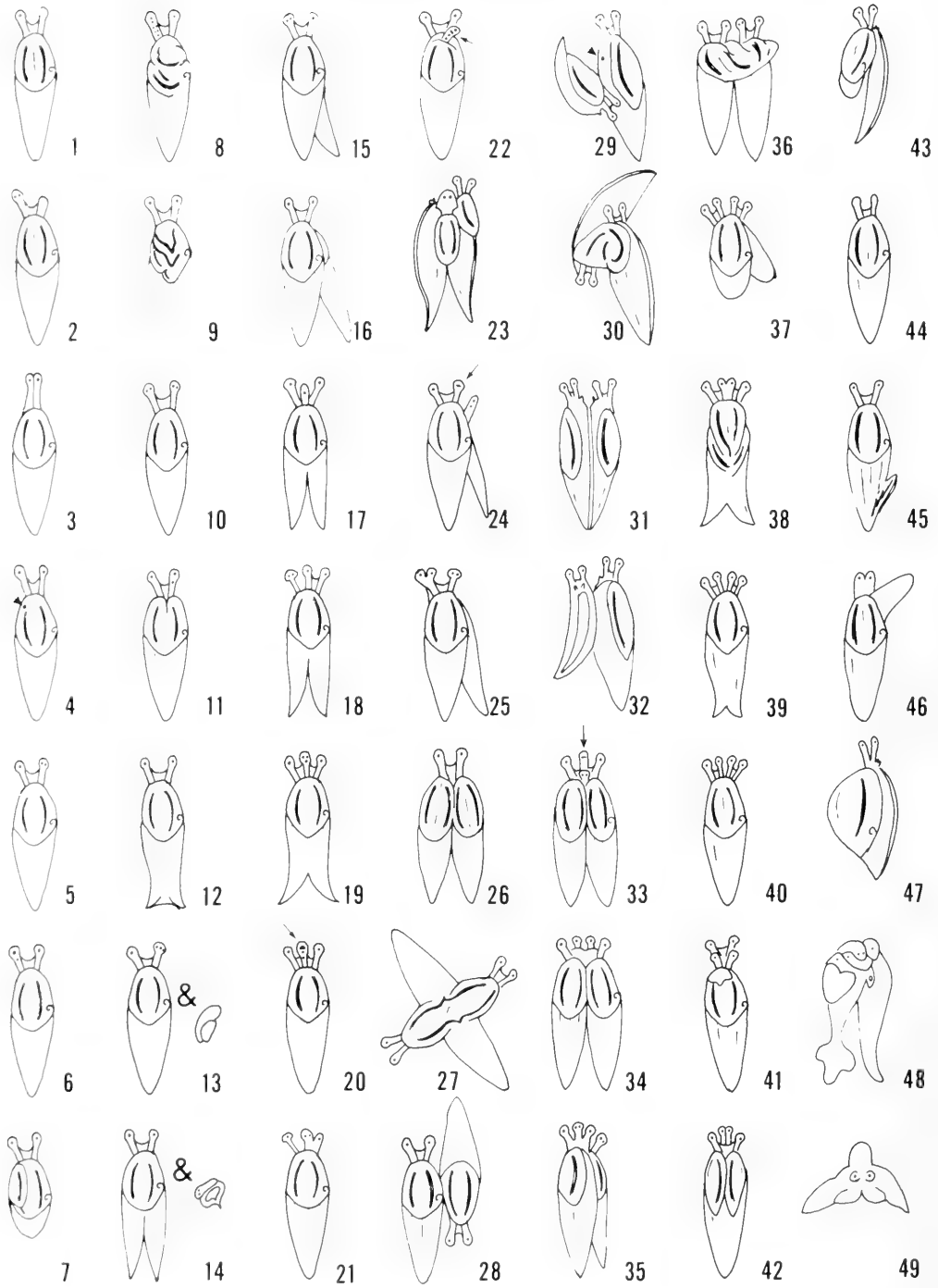
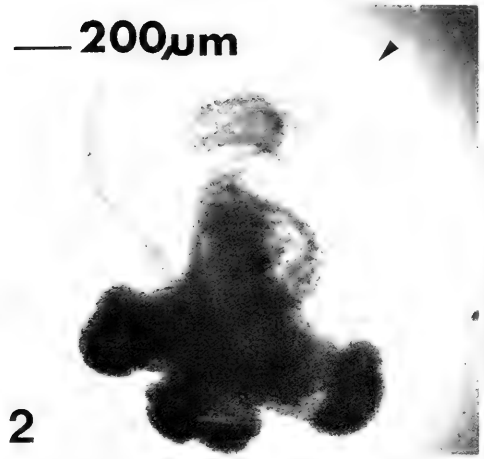
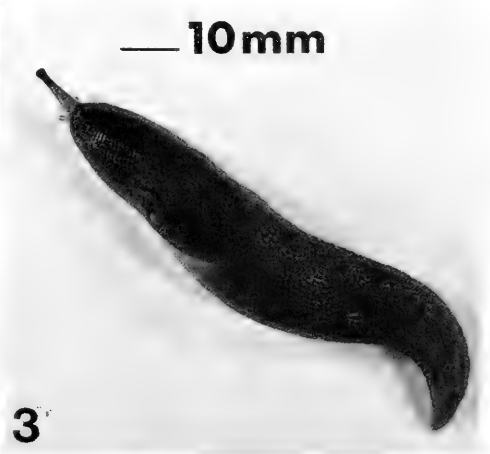
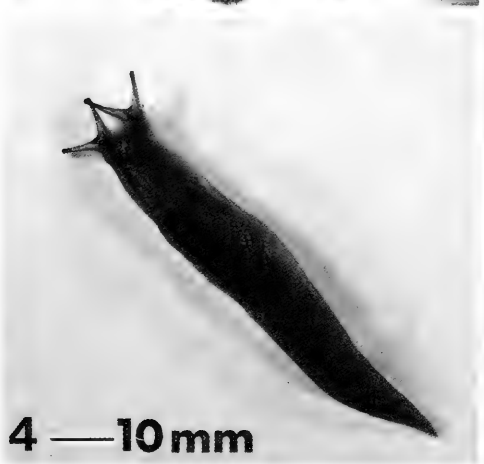
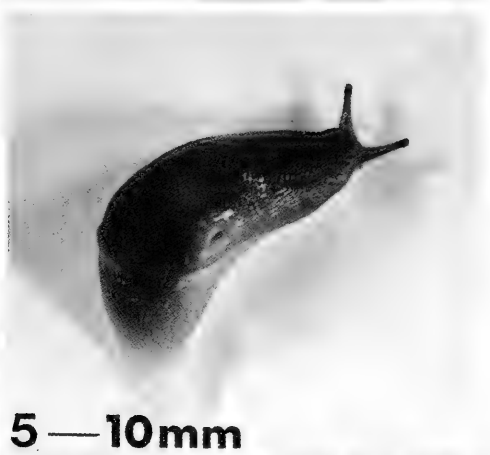


FIG. 1. *Lehmannia valentiana* conjoined twins and other anomalies. (Arrows indicate examples of supernumerary eyes, cyclopia, unusual placement of eyes.)









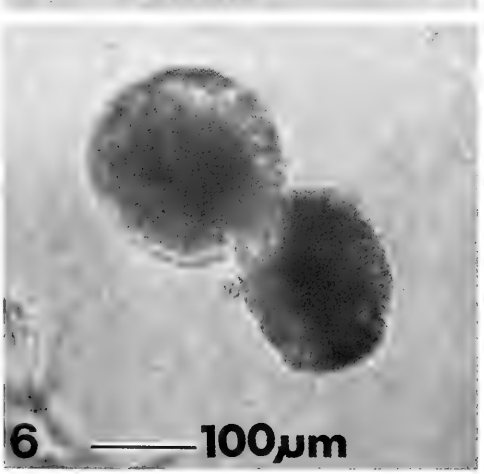


FIG. 2. Representative sample of unusual morphology in *L. valentiana* with an embryo showing arrested development (arrow indicates posterior sac). FIG. 3. Adult *L. valentiana* with fused tentacles. FIG. 4. Two-headed one-tailed conjoined twin. FIG. 5. Mantle hump, short tail adult animal. FIG. 6. Example of double ova representative of close doublet, some of which develop into conjoined twins. FIG. 7. Unusual conjoined twin oriented at 180°. (Photos 3, 4 and 5 taken by J. Coggins.)

TABLE 1. The incidence of conjoined twins and other defects at the time of hatching recorded in *Limax maximus, Deroceras reticulatum,* and various *Lehmannia valentiana* groups.

Group	Number times eggs collected	Complete embryonic development* (no.)	Conjoined twins (%)	Other defects (%)	Total abnormalities (%)
Deroceras reticulatum	17	1562	0.0	0.1	0.1
Limax maximus	14	1791	0.0	0.7	0.7
Lehmannia valentiana (orig. group)	18	371	6.7	3.2	9.9
L. valentiana (1st generation)	50	4201	1.4	2.4	3.8
L. valentiana (2nd generation)	25	7198	3.1	0.7	3.8
L. valentiana (3rd generation)	4	633	1.7	0.3	2.1
L. valentiana (Kentucky-wild)	6	22	4.5	4.5	9.1
*L. valentiana*** (hybrid)(N = 2)	5	256	1.6	0.7	2.3
L. valentiana (isolate)*** (N = 1)	3	7	14.3	0.0	14.3

*Complete embryonic development was determined by the appearance of pigmentation and the disappearance of embryonic features, such as the pedal lobe.

**These two animals were obtained from *L. maximus* eggs in a breeding experiment between *L. maximus* and *D. reticulatum* (Mason, 1985).

***This was the only one of three animals that were isolated from birth that produced any eggs.

twin, a partially developed foot and body, is shown in number 45.

The lateral views (numbers 43 and 47) show mantle deformities that may not be directly associated with twinning in all cases. An epidermal invagination, forming a pouch, separates the mantle and viscera from the foot (number 43). In number 47, the animals were abbreviated in the antero-posterior axis. The viscera and mantle were elongated dorsoventrally as if torsion had only partially occurred or had occurred at an abnormal angle.

The abbreviated bodies (numbers 7 and 37) are both the result of conjoining as evidenced by morphological duplication of parts.

Fig. 1 is fairly thorough in the presentation of anomalies with these exceptions:

1. The numerous variations of dorsolateral joining are not shown (all antero-posterior joining variations are shown.

2. The morphology of animals that did not reach hatch status, other than numbers 48 and 49, were not recorded.

3. No animals with mouth deformities, a frequent but fatal occurrence, are shown.

Capsule doubling

Conjoined twinning has been attributed to fusion of two or more zygotes in the same capsule (Crabb, 1931; George, 1958, Bigus, 1981). The distance between two *L. valentiana* zygotes in the same quadrant of a capsule was measured using a light microscope micrometer. The average distance between the two zygotes (43 capsules) was 16.2 μm, with a range of 0.0 μm to 68.8 μm at × 400 magnification. Fig. 6 is an example of the 0.0 μm distance, where no separation is discernible. If doubled, the two zygotes are frequently in the same quadrant of a capsule in both *D. reticulatum* and *L. valentiana,* but seldom in *L. maximus.*

By segregating *L. valentiana* capsules containing close doublets (0.0 μm separation), we determined that conjoined twinning did not occur unless the zygotes were extremely close, a finding substantiated by others (George, 1958; Crabb, 1931). When we dissected slugs while they were laying eggs, we found the closeness of the double zygotes was present throughout the unlaid capsules

TABLE 2. The incidence of conjoined twins and other defects at the time of hatching recorded for various *L. valentiana* abnormal groups.

Group	Number times eggs collected	Complete embryonic development* (no.)	Conjoined twins (%)	Other defects (%)	Total abnormalities (%)
L. valentiana fused tentacles (N = 6) (Fig. 3)	26	577	4.8	2.1	6.9
Two-head, one-tail (N = 4) (Fig. 4)	10	118	1.7	2.5	4.2
Mantle hump-short tail (N = 4) (Fig. 5)	13	381	0.3	0.5	0.8
Eye/tentacle defect (N = 3)	15	239	0.4	1.3	1.7
Paired close doublets** (N = 8)	27	959	6.1	3.1	9.2

*Complete embryonic development was determined by the appearance of pigmentation and the disappearance of embryonic features, such as the pedal lobe.

**Paired close doublets were animals obtained by raising animals which had developed as two animals in one egg capsule. Closeness was determined by whether there was a measurable distance between the fertilized ova prior to first cleavage. If the doublets were "close," the distance was not measureable at × 100 magnification. Those close doublets which resulted in conjoined twins were not used in this breeding experiment.

enclosed in the oviduct. Fusion or whatever mechanism was responsible for conjoined twinning was occurring prior to cleavage and might be occurring prior to oviposition.

Conjoined twinning in *L. valentiana* is not the result of incomplete fission of the first or subsequent cleavage stages. This was determined by segregating all capsules containing double or multiple zygotes from singlets at the time of oviposition. No conjoined twins were recovered from the singlet capsules.

The incidence of conjoined twinning and other anomalies was recorded for *L. maximus, D. reticulatum* and various *L. valentiana* groups (Table 1). No conjoined twins were observed for *L. maximus* and *D. reticulatum.* The "other defects" were generally mouth deformities, i.e. extrusion of the buccal cavity. No duplication of parts was observed. The *L. valentiana* original colony's percentage of conjoined twins is higher than that of the following generations, but similar to the Kentucky population, and the paired close doublets.

Of the three animals isolated from birth, only one produced eggs. This animal laid 22 eggs when it was 8.3 months old (a non-isolated or colony animal frequently lays 100 or more eggs at a time beginning at 4.5 months of age). Of those 22 eggs, 6 produced normal animals and one a conjoined twin, while the capsules containing multiple ova did not develop. The late onset of egglaying and the high percentage (63.6%) of multiple ova in the capsules (Table 3) may represent a "last ditch" effort to reproduce. One of the three animals lived 15.5 months without laying eggs. That is the longest a *L. valentiana* has lived in our laboratory.

The results of mating several groups of animals possessing similar abnormalities (Fig. 3, 4, and 5) are recorded in Table 2. Although conjoined twins and/or other defects occurred in all groups, the morphology of the offspring did not match that of the parents, i.e. neither of the two conjoined twins produced by the "two-head, one-tail" parents was similarly joined.

Considerable disparity exists in the percentage of conjoined twins produced by the five groups of abnormal parents. The "mantle hump-short tail" and "eye/tentacle defect" groups' percentage of conjoined twins was less than a third that of any of the other groups. The "paired close doublets" produced the most conjoined twins, but not higher than the original *L. valentiana* colony (Table 1).

The total number of doublets and multiple egg capsules in various groups was tested using Chi-square contingency tables at the $P_{99.5}$ significance level (Table 3). No significant difference in the proportion of total doublets and multiples was found between the *D.*

TABLE 3. The incidence of egg capsules containing more than one ovum recorded in *Deroceras reticulatum*, *Limax maximus* and other *Lehmannia valentiana* groups. Singlet = animal originated in capsule containing one ovum. Doublet = animal originated in capsule containing two ova.

Group	Number times eggs collected	Capsules (no.)	Doublets (%)	Multi (%)	Total (%)
Deroceras reticulatum	9	1142	6.9	1.0	7.9
Limax maximus	16	1716	5.1	2.2	7.3
Lehmannia valentiana (mixed lab colony)*	26	5330	9.6	1.3	10.9
L. valentiana (Kentucky-wild)	6	139	10.1	0.7	10.8
L. valentiana (singlet colony)**	7	1565	4.0	0.3	4.3
L. valentiana (doublet colony)***	7	1608	9.8	1.3	10.4
L. valentiana (paired close doublets)**** (4 pair, N = 8)	27	2593	24.5	2.3	26.8
L. valentiana (paired singlet)***** (3 pair, N = 6)	5	287	3.8	1.1	4.9
L. valentiana (selfing [singlet] individual) (N = 1)	3	22	9.1	63.6	72.7

*Colony consisted of both singlets and doublets.
**Colony consisted only of animals which had originated as single ova in one capsule.
***Colony consisted only of animals which had originated as double ova in one capsule.
****Two animals which had originated as double ova in one capsule were paired with each other and isolated from other animals. Four pair of close doublets were used.
*****Two animals which had originated as single ova in capsules were paired with each other and isolated from other animals. Three pair of singlets were used.

reticulatum and *L. maximus* colonies (df = 1), between the *L. valentiana* singlet colony and paired singlets (df = 1), or between the *L. valentiana* mixed, Kentucky, and doublet colonies (df = 2). A significant difference was found between the *D. reticulatum, L. maximus* and *L. valentiana* mixed colonies (df = 2), the *L. valentiana* paired singlets and the mixed colony (df = 1), and the *L. valentiana* paired close doublets and mixed colony (df = 1).

If the *L. valentiana* paired close doublets are excluded, the proportion of multiple (more than two per capsule) ova was not significantly different in the other *L. valentiana* groups (df = 4). The variation between these groups seems to be dependent on the number of doublets produced.

L. valentiana can self-fertilize (Table 1), but not consistently. The rate of doubling in the selfing individual is similar to that in the mixed colony, i.e. 9.1%. The percentage of multiple ova capsules is 63.6%, additional evidence of the independence of doubling and multiples. The selfing animal was not statistically tested with the other groups due to the small sample number.

DISCUSSION

Duplication of parts

Eye and tentacle defects without evidence of other morphological duplication do not initially imply conjoined twinning as a causal agent. Extra eyes, extra heads and other duplications have been chemically induced in insect embryos (Walton *et al.,* 1983). Separation of molluscan embryos after first or second cleavages can result in the absence of eyes (and tentacles) in one of the halves or both, or both halves may each have two eyes (Cather *et al,* 1976). The anterior end of an

animal is the most susceptible to agents that inhibit development (Newman, 1917).

The frequent occurrence of supernumerary or absent eyes in *L. valentiana* animals suggests that two initially conjoined zygotes may separate after the eye anlage has differentiated. This event might occur after the first quartette of micromeres is produced as these cells give rise to the cerebral ganglia, cephalic eyes and tentacle (Verdonk, 1979). This event, however, would seem to account only for the presence of four eyes, i.e. two from each zygote. Tweedell (1953) noted that dissymmetries might arise if a structure is formed from a fraction of the total mass. Such a fraction might be embryonic cells detached from one twin and incorporated into the other.

If random fusion were the causal mechanism, one would expect conjoined twins to resemble those shown in numbers 28, 30 or 31 of Fig. 1, where the twins are equal and complete. However, Hess (1971) claims that in experimentally produced gastropod single-egg twins, the material originally included in only one egg is capable of developing three or four tentacles or eyes instead of the normal single pair. The presence of eight eyes on animals 8 and 22 (Fig. 1) parallel Hess' observation. Although *L. valentiana* conjoined twins could arise by fusion of two zygotes, an incomplete and unequal fission of a fertilized ovum is not completely ruled out. However, in the latter case, if Hess is correct, animals with eight eyes are theoretically unlikely.

Body alignment

The frequency of anterior-to-anterior alignment seems unusual in *L. valentiana* conjoined twins if orientation occurred randomly. Animal-vegetative polarity is established during oogenesis (Verdonk, 1979). The animal pole protrudes into the lumen of the gonad in spiralian oogenesis (Huebner & Anderson, 1976). Raven (1967) argued that even the symmetry and dorsoventrality of the future embryo is imprinted on the egg cortex by the surrounding gonadal follicle cells.

We posit several mechanisms to account for the preferential anterior-to-anterior conjoining observed: (1), the oocytes are fused in the gonad; (2), anterior-posterior fusion reduces the viability of the embryo, i.e. death occurs before we could determine alignment; (3), fusion induces a polarity change; (4), conjoined twins do not arise as a result of fusion, but by some other mechanism.

Fusion of individual cells, whether germ or somatic, is not easily achieved despite the normal occurrence of early embryonic junctions between blastomeres. In separated blastomeres, ". . . very tight coupling resumes only if the cells are brought back together quickly and in the original orientation" (Powers & Tupper, 1977). Most centrifuge experiments aimed at fusing close zygotes or oocytes yield less than satisfactory results (including our own). N. H. Verdonk (personal communication, 1982) noted that whereas multiple ova egg capsules were found in nearly all mollusk groups,

> "Spontaneous fusion of eggs or embryos is very exceptional even when many eggs are stored in the same capsules. The reason is that most eggs are surrounded by a vitelline-membrane and as soon as the embryo comes out of this membrane it starts turning around."

Verdonk suggested that the mechanism underlying the relatively high rate of "germ fusions" in *P. acuta* (Bigus, 1981) is a missing or defective vitelline membrane, which would allow fusion to occur.

The *Lymnaea stagnalis* embryo leaves the vitelline membrane approximately 32 hr. after the first cleavage and begins turning (Verdonk, personal communication, 1983). *L. valentiana* embryos are turning around at the time the first polar body is extruded (usually within one hour after oviposition). Rotation is easily observed by watching the polar body seem to appear and disappear. If the *L. valentiana* zygote turns around after leaving the vitelline membrane, as Verdonk observed in *L. stagnalis,* then *L. valentiana* zygotes have left the vitelline membrane prior to extrusion of the first polar body, or the vitelline membrane may be absent or defective.

The actual number of *L. valentiana* conjoined twins may be under-represented in Table 1 since a number of the terata listed under "other defects" may originate as doublets or conjoined twins. Therefore, especially in the early data, a problem of "fuzzy-sets" (Root-Bernstein, 1983) exists between the *L. valentiana* categories "conjoined twins" and "other defects."

The number of conjoined twins and consequently total abnormalities is higher for the original and Kentucky colonies and the paired close doublets than in the other groups. Since the paired close doublets were selected with

the intent of increasing the doubling and consequently the conjoined twinning, this higher rate was anticipated. However, other explanations must account for the higher rate in the original and Kentucky colonies.

In the original *L. valentiana* colony, conjoined twinning data were not taken for the first four months of egg laying since we did not know it was occurring. The Kentucky colony slugs oviposited in late autumn and died soon thereafter. Data, in both cases, were, therefore, from the later stage of fertility. If one does not have data from the first two-thirds of fertility, the last third may appear inflated. Bigus (1981) noted that both doubling and zygote fusion in *P. acuta* occurred *only* during the last third of fertility. This is not true in *L. valentiana* since many early clutches contain both conjoined twins and doublets.

The original *L. valentiana* colony differs from the Kentucky colony in that whereas the total abnormalities produced is similar, the original colony produced more conjoined twins while the Kentucky colony produced more "other defects."

Since the original colony was a first generation hybrid, the trait may have been attenuated in subsequent generations. However, the two *L. valentiana* later obtained by hybridization did not produce a higher rate of conjoined twins.

Minimally, the *L. valentiana* conjoined twinning rate was greater than 1.0% in *all* eggs collected with the exception of the "mantle hump-short tail" and "eye/tentacle defect" matings (Table 2). This 1.0 % rate is 50 times greater than the rate Bigus (1981) observed in *P. acuta* and the other mollusks cited. More importantly, *L. valentiana* data only include animals that developed to the hatch stage and were viable. We conclude that the initial rate of conjoined twinning is even higher. None of the conjoined twins observed by Bigus or others reached hatch status.

The production of conjoined twins by an isolated *L. valentiana* animal demonstrates the possibility of a founder animal producing a population containing the trait, an important consideration in a hybrid animal.

The low rate of abnormalities, especially conjoined twins produced by the "mantle hump-short tail" and "eye/tentacle defect" pairings is difficult to explain. Since most "eye/tentacle defect" animals originated as doublets, one would expect the trait to be expressed with a frequency equivalent to that of the other colonies. These pairings, however, do demonstrate the fertility of animals with several types of abnormalities.

The results of selective breeding of various *L. valentiana* groups indicates that the occurrence of doublets in egg capsules is inheritable (Table 3). This finding contradicts both Crabb (1931) and Bigus (1981). Using the *L. valentiana* mixed laboratory colony as standard, the rate of doubling is 9.6 %. The singlet colony and paired singlets rate of doubling is less than half that rate (4.0 and 3.8 % respectively). The colony consisting only of animals which originated as doublets had a rate of doubling consistent with that of a mixed colony, possibly an indication that mating among these animals was random, i.e. twins did not mate with their capsule siblings. However, one would expect the doublet colony animals to produce more doublets than the unselected colonies if there were complete penetrance of the trait.

The ability to inherit the doubling trait is also demonstrated by the results of the paired close doublet matings, i.e., 24.5 % of their offspring were also doublets. The difference in doubling rate between the paired close doublets (24.5 %) and the doublet colony (9.8 %) may be attributable to the selection process. Colony doublets were obtained from capsules containing two ova but not necessarily closely apposed ova as was the case in the paired close doublets. More than one mechanism may exist for doubling, one based on an anatomical defect such as described by C. P. Raven (personal communication, 1981) and one unknown. The cause of doubling and/or conjoined twinning may be inherent in the zygote or a result of the reproductive environment.

As a hybrid, *L. valentiana* may be exhibiting a mixture of the developmental pathways of the putative parental species *D. reticulatum* and *L. maximus,* neither of which produced conjoined twins. Rachootin & Thomson (1981) noted that ". . . a mixture of related but distinct developmental pathways might produce adaptively interesting novelties, which on occasion are assimilated."

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THE FEEDING OF TERRESTRIAL SLUGS IN RELATION TO FOOD CHARACTERISTICS, STARVATION, MATURATION AND LIFE HISTORY

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ABSTRACT

Food consumption by adult *Deroceras reticulatum* was measured as dry weight, wet weight and volume of food eaten. Consumption varied least among diets when wet weights were compared. Slugs ingested one to four meals daily. The number, duration and size of meals and the interval between them varied widely among individuals, with the type of food and with the duration of starvation.

Starved adults showed no compensation following starvation when either consumption or growth was considered, although the number and frequency of meals increased with increasing deprivation. Similar results were found for the much larger, longer-lived species *Limax maximus,* suggesting that differences in life history tactics were not involved. Immature *D. reticulatum,* however, showed strong compensatory growth (and presumably feeding) following starvation. Degrowth was a key response to starvation which may explain why gastropods do not accumulate appreciable reserves of lipids. Slugs apparently are capable of long-term regulation during the growth phase of their life cycle. During reproduction, however, adults lost weight even when fed, and compensation was lacking.

Key words: slugs; *Deroceras reticulatum; Limax maximus;* feeding regulation; starvation.

INTRODUCTION

Terrestrial molluscs are major consumers and decomposers in natural, agricultural and horticultural communities (Godan, 1983). Consequently, there is considerable literature concerned with their food consumption (see Rollo, 1987). Understanding feeding is particularly important since control is mainly by poisoned baits (Wright & Williams, 1980). Senseman (1978) and Reingold & Gelperin (1980) examined control of ingestion for particular meals, but there is almost nothing known about the daily frequency and duration of meals as influenced by food characteristics.

Many invertebrates respond to starvation or malnutrition by compensatory mechanisms such as increased feeding rates (Waldbauer, 1968; Gelperin, 1971; Barton-Browne, 1975; Slansky & Scriber, 1985). Some gastropods, however, may lack such compensatory abilities (Susswein & Kupfermann, 1975a, 1975b; Senseman, 1977). Food quantity is rarely limiting for general herbivores such as snails or slugs, but unfavourable weather may restrict foraging (Richter, 1976; Rollo, 1982), or require aestivation (Schmidt-Nielsen *et al.,* 1971; Jaremovic & Rollo, 1979; Rollo, 1982). Whether molluscs compensate to offset such setbacks has important implications for their growth, reproduction and population dynamics. Calow (1975a, 1975b) found post-starvation compensation in aquatic snails, but terrestrial slugs have not been studied in this regard. The present study characterized the daily feeding pattern of the terrestrial slugs *Deroceras reticulatum* (Müller) and *Limax maximus* L. on various diets. Post-starvation responses were also examined to determine if long-term regulation occurred.

METHODS

Daily feeding pattern and the starvation response

Adult *D. reticulatum* were collected from fields in Hamilton, Ontario in October and November (part of their normal reproductive period). Slugs were housed individually in glass jars 6 cm deep and 5 cm in diameter. Each jar contained 2 cm of moistened vermiculite which maintained high humidity but was never eaten. To ensure entrainment of the animals' circadian rhythms (Rollo, 1982), slugs were housed in environmental cham-

bers with a light:dark cycle of 16:8 h, and a temperature of 18°C for two weeks prior to the experiments. During entrainment the slugs were fed lettuce. Jars were cleaned and the vermiculite was replaced weekly. Slugs were weighed and placed in clean jars prior to experiments. Most faecal strings were deposited during the light period, and these were removed prior to the dark period to prevent coprophagy. Adults were assigned so that their mean weight was similar among treatments (see Table 1). For calculating dry weight, all animals were assumed to be 89% water, based on a sample of 10 individuals.

Cucumber (*Cucumis sativus* L. var. *anglicus*) was mainly used to examine the influence of starvation on feeding. Ingestion is also influenced by physical characteristics of food such as hardness (Senseman, 1978). Therefore a harder food, carrot root (*Daucus carota* L.), was also studied. Both foods were highly palatable to *D. reticulatum*. There is little standardization in the way that consumption rates or food characteristics are measured which makes comparative studies difficult. Therefore, wet weight, dry weight and volume eaten were all evaluated.

Feeding by *D. reticulatum* on cucumber was monitored following starvation for 16, 40, 100 and 220 h. Similar observations were made when carrot was fed to slugs starved 16 or 100 h. For each experiment, 20 1 cm^3 cubes of carrot or cucumber were dried at 60°C to constant weight. This sample provided estimates of hydration and dry weight-to-volume relationships. Each slug was given a pre-weighed cube of appropriate food just before the dark period, and the behaviour of each individual was recorded at 0.5 h intervals until the next light period. Whenever a slug completed a meal, the remaining food was removed and a new pre-weighed food cube was supplied.

The food remaining following a meal was dried to constant weight at 60°C. Consumption was calculated by subtracting the dry weight of the remainder from the estimated dry weight of the original food. The volume and wet weight eaten were calculated by multiplying the dry weight ingested by the appropriate conversion factors. Slugs that oviposited were excluded from the analysis because this activity had a longer duration and higher priority than feeding. Slugs ate distinct meals separated by several hours. Consequently a 0.5 h observation interval was sufficient to distinguish meals.

Snails may reduce their metabolism or aestivate during starvation or dehydration (Heeg, 1977). If slugs respond similarly, they could require time to become fully active and so their initial feeding could be relatively low. Consequently, feeding of slugs starved 16 or 100 h was observed for two consecutive days after being fed cucumber.

Life cycle considerations

Initial experiments did not detect compensatory feeding following starvation. Consequently, several additional hypotheses were tested. Other preliminary experiments suggested that starved adult *D. reticulatum* continued ovipositing which suggested that they were irreversibly committed to reproduction. Most adults lost weight even when fed, suggesting that regulatory mechanisms such as compensatory feeding may be absent and somatic support reduced. Compensatory mechanisms might still occur in juveniles, but it was difficult to accurately measure ingestion by small slugs. If compensation occurs, however, it should be reflected in growth rate. Young *D. reticulatum* (mean wet weight of only 66 mg), were collected from burdock plants (*Arctium minus* (Hill) Bernh.) in June. The animals were starved for 2 days to evacuate their guts, and then weighed. Eighteen slugs were fed fresh burdock leaves at 18°C, 100% R.H. and with a light:dark cycle of 16:8 h. Another 18 slugs were starved for 36 days in identical conditions (to 50% mortality) and then fed burdock. Animals in both treatments were weighed every 3 to 4 days and slugs that died were omitted from the analysis.

The growth rates of fed and starved adult *D. reticulatum* were also examined to see if their growth response was consistent with their feeding behaviour. Adult slugs were collected from burdock rossettes in the fall and were treated identically to the juveniles (32 starved adults, 18 fed adults). This experiment was terminated after 20 days and any slugs that died were omitted from the analysis.

Life history considerations

D. reticulatum is an annual and may sacrifice the parent to augment reproduction. Consequently compensatory feeding could be abandoned in mature animals. This hypothesis was tested by examining the response of a related species with greater parental investment. *L. maximus* lives 2 to 3 years and

TABLE 1. Consumption of adult *Deroceras reticulatum* on carrot or cucumber following various periods of starvation in a photoperiod of light:dark 16:8 h and temperature of 18°C. FD = The percentage of dry matter in the food. SL = The mean dry pre-starvation weight (mg) of slugs in the treatment. n = The number of slugs on which the means are based. Tot. = The mean daily feeding by an individual slug during the 8 h dark period.

Food	Starvation period (h)	Meal	Consumption (means and S.E.) g wet food/ g wet slug		cm^3/ g dry slug		g dry food/ g dry slug		Percent feeding
Carrot	16	1	0.215	0.029	1.536	0.214	0.234	0.033	100.0
FD = 11.96		2	0.263	0.045	1.872	0.322	0.286	0.049	81.3
SL = 0.0740		3	0.242	0.043	1.723	0.304	0.263	0.046	25.0
n = 16		Tot.	0.489		3.488		0.532		
Carrot	100	1	0.224	0.036	1.596	0.254	0.243	0.039	100.0
FD = 11.96		2	0.225	0.037	1.606	0.267	0.245	0.041	62.5
SL = 0.0861		3	0.434	0.0	3.093	0.0	0.472	0.0	6.3
n = 16		Tot.	0.392		2.793		0.426		
Cucumber	16	1	0.465	0.077	3.366	0.560	0.126	0.021	100.00
FD = 2.98		2	0.379	0.059	2.742	0.543	0.103	0.020	63.2
SL = 0.0574		3	0.391	0.059	2.831	0.434	0.106	0.016	21.1
n = 19		4	0.218	0.0	1.581	0.0	0.059	0.0	5.3
		Tot.	0.798		5.777		0.216		
Cucumber	40	1	0.303	0.048	2.805	0.449	0.104	0.017	100.0
FD = 3.79		2	0.258	0.038	2.387	0.356	0.089	0.013	78.6
SL = 0.0613		3	0.405	0.192	3.751	1.782	0.139	0.066	21.4
n = 14		Tot.	0.593		5.484		0.204		
Cucumber	100	1	0.393	0.021	2.843	0.154	0.107	0.006	100.0
FD = 2.98		2	0.238	0.022	1.726	0.159	0.065	0.006	76.5
SL = 0.0742		3	0.228	0.039	1.652	0.281	0.062	0.011	29.4
n = 17		4	0.261	0.0	1.888	0.0	0.071	0.0	5.9
		Tot.	0.657		4.759		0.179		
Cucumber	220	1	0.319	0.046	2.399	0.347	0.075	0.011	100.0
FD = 3.17		2	0.210	0.032	1.580	0.239	0.049	0.007	63.6
SL = 0.0432		3	0.131	0.022	0.988	0.162	0.031	0.005	36.4
n = 11		4	0.145	0.045	1.092	0.341	0.034	0.011	18.2
		Tot.	0.527		3.962		0.124		

grows to 5 to 20 g compared to only 0.5 to 1.5 g for *D. reticulatum*. If life history tactics are important, adults of *L. maximus* should strongly compensate for starvation.

L. maximus were collected from a local deciduous woodland in Hamilton, Ontario (a new locality record) in October. The slugs were starved for 24 h to clear their guts and then weighed (range = 3.9 to 15.6 g live weight, n = 11). The experiment was carried out identically to that for *D. reticulatum*, except that cubes of potato tuber (*Solanum tuberosum* L.) were used as food, and only dry weight consumption was considered. Potato was chosen because it was highly palatable to this species, whereas the acceptability of carrot or cucumber varied among individuals. Nocturnal feeding was monitored following 24 h starvation and, using the same animals, after 288 h starvation. The potato used following 24 h of starvation was 78.98% water and that for the 288 h starvation period was 78.19% water.

RESULTS

Daily feeding pattern and the starvation response

To calculate mean meal sizes, daily consumption and the likelihood of successive meals, only animals that fed were considered (Table 1). Slugs starved 16 or 40 h all ate but

TABLE 2. Feeding of *Deroceras reticulatum* on the second night following 16 h or 100 h of starvation. FD = The percentage of dry matter in the food. SL = The mean dry weight of slugs (mg). n = The number of slugs in the treatment.

Food	Initial starvation period (h)	Meal number	Consumption (g wet food/g wet slug) Mean	S.E.	Percent feeding
Cucumber	16	1	0.336	0.098	100
FD = 3.34		2	0.322	0.123	29.4
SL = .0574		Total	0.373		
n = 17					
Cucumber	100	1	0.181	0.031	100
FD = 3.34		2	0.321	0.168	38.5
SL = .0861		3	0.166	0.0	7.7
n = 13		Total	0.317		

TABLE 3. Feeding of *Limax maximus* on potato tubers following starvation for 24 h or 288 h (18° C, light:dark, 16:8, R.H. 100%).

Treatment	Mean weight of slugs (dry g)	Number of animals	Feeding (mean +/− S.E.) (g dry food/g dry slug) Meal 1	Meal 2	Daily Consumption
Starved 24 h	0.908	11	0.405, 0.057 n = 11	0.237, 0.074 n = 5	0.583, 0.096 n = 11
Starved 288 h	0.859	11	0.247, 0.050 n = 11	0.081, 0.021 n = 10	0.321, 0.051 n = 11

only 90% of slugs starved 100 h and 85% of slugs starved 220 h ate. Slugs starved longer than 40 h also responded slowly to the presence of food. Starvation had little influence on the percentage taking a second meal of cucumber (63%–79%), but the probability of eating a third or fourth meal increased markedly with prolonged starvation (Table 1). Despite this, there was a progressive decline in daily consumption and meal size with increasing deprivation, no matter how consumption was measured.

D. reticulatum ate 1 to 4 meals of cucumber per night. The first meals were the largest (.303 to .465 g wet food/g live slug), and fourth meals were relatively small (.145 to .261 g wet food/g live slug) (Table 1). *D. reticulatum* starved 220 h ate 57% as much dry food/day as individuals starved 16 h (66% on a wet weight basis) but they were still 88.7% of their original wet body weight. Thus, feeding was reduced more than expected from loss of body mass. *D. reticulatum* starved 100 h also ate less carrot than those starved 16 h, although meal size was not reduced. No more than three meals of carrot were eaten per night and meal frequency declined with longer deprivation. Nearly twice as much dry carrot as cucumber was eaten per day but at least 1.6 times more cucumber than carrot was ingested in terms of wet weight or volume. For individual meals, the least variation between the diets was obtained when wet weight was measured. Most of the difference in daily consumption was related to the number of meals.

Although some meals were shorter than the 30 min observation period, it was possible to discern patterns in the duration and frequency of meals. Nearly all meals of cucumber took less than 30–45 min. Initial meals were the longest whereas most fourth meals were completed in less than 30 min. Whereas slugs usually fed and then returned to their resting position, those starved 100 or 220 h spent 60 to 90 min resting on the food. Slugs took con-

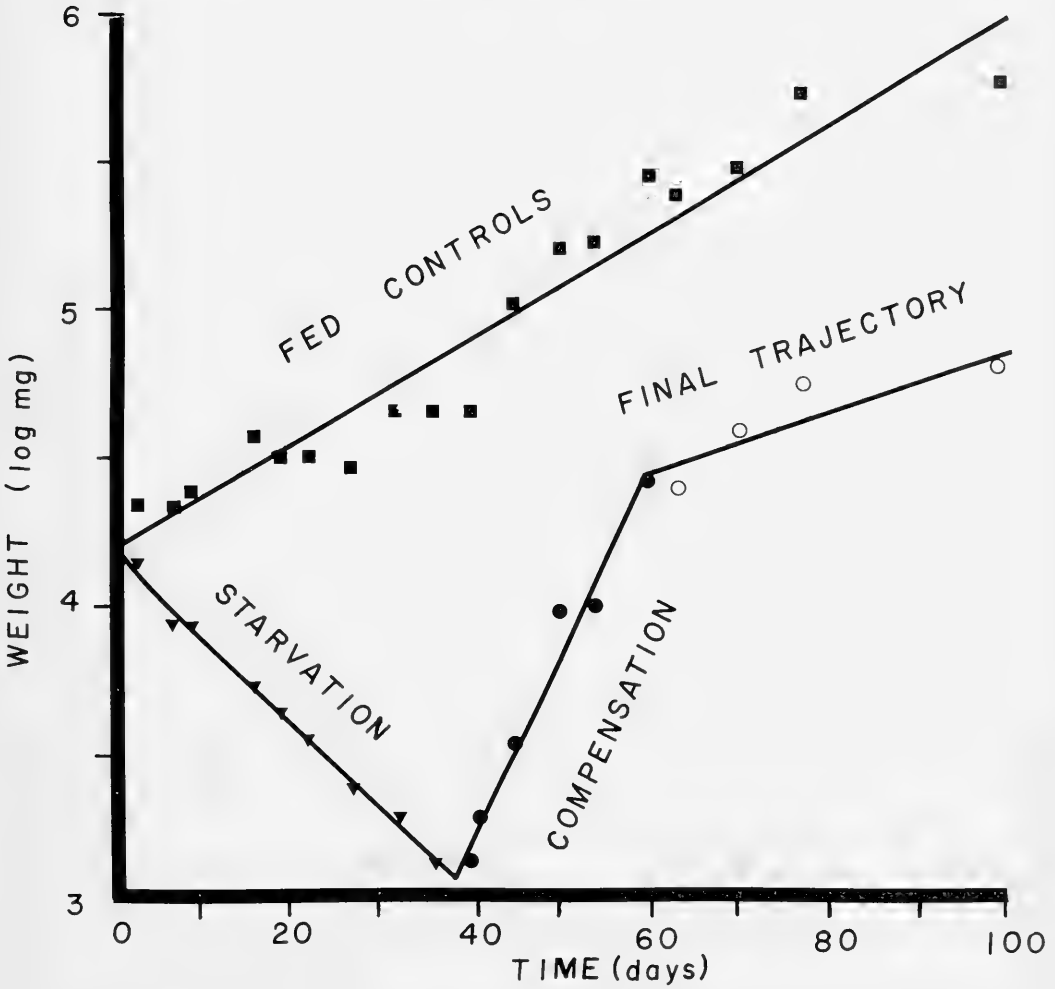

FIG. 1. Growth of juvenile *Deroceras reticulatum* at 18°C when fed leaves of burdock, or starved for 36 days prior to feeding. The regression equation for fed controls (■) was:
$\text{Log}(Y) = 4.175 + 0.01805(X)$ $r^2 = 0.93$, $n = 18$, $p < 0.001$.
The best-fitting equation for degrowth of starved slugs (▼) was:
$\text{Log}(Y) = 4.187 - 0.0291(X)$ $r^2 = 0.99$, $n = 9$, $p < 0.001$.
During the compensatory period (●) the regression equation was:
$\text{Log}(Y) = 0.7517 + 0.0615(X)$ $r^2 = 0.97$, $n = 6$, $p < 0.002$.
The following final growth trajectory (○) had the equation:
$\text{Log}(Y) = 3.8051 + 0.01066(X)$ $r^2 = 0.82$, $n = 5$, $p < 0.10$,
where Y = log wet weight (mg) and X = days.

siderably longer to eat carrot. Average durations ranged from less than 30 up to 90 min, but first meals usually required nearly 1 h.

The interval between the first and second meals of cucumber was the longest, but decreased with longer starvation. Slugs starved 16 h ate their second meal after about 4 h, whereas slugs starved 220 h ate again in only 2 h. The interval between meals 2 and 3 was usually 2.5 h. Slugs starved 220 h, however, ate again in about 1.5 h. In all cases meals 3 and 4 were only 1 or 2 h apart. Animals deprived 16 h and then fed carrot ate again about 2.5 h following their first feeding. Those starved 100 h, however, took 3.5 to 4 h to initiate a second meal. In both treatments the

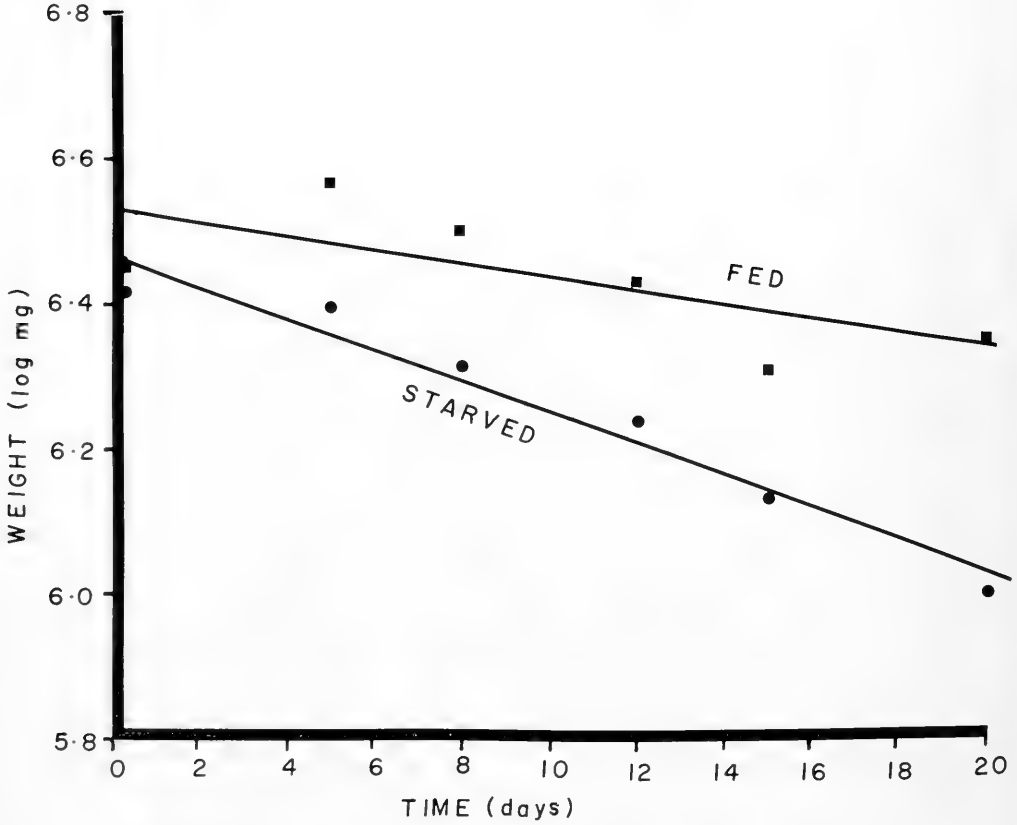

FIG. 2. Growth of adult *Deroceras reticulatum* when fed leaves of burdock or starved at 18°C. The best-fitting equation for the fed animals (■) was:
$\text{Log}(Y) = 6.5277 - 0.009446(X)$ $r^2 = 0.51$, $n = 6$, $p < 0.50$.
For starved animals (●) the regression equation was:
$\text{Log}(Y) = 6.4646 - 0.021795(X)$ $r^2 = 0.95$, $n = 6$, $p < 0.005$,
where Y = log wet weight (mg) and X = days.

interval between the second and third meals was about 2 h.

Next day feeding

Feeding on the second day following reestablishment of food was greatly reduced, regardless of the deprivation period. Ten percent of the slugs did not feed, individual meals were smaller, and the likelihood of taking consecutive meals was markedly reduced (Table 2). Slugs initially starved 16 h never took more than two meals of cucumber on the second day of feeding. Daily wet weight consumption was only 46.7% that of the first day for slugs deprived 16 h and 48.2% of first-day feeding for those starved 100 h (Table 2).

Life cycle considerations

Juvenile *D. reticulatum* fed burdock grew rapidly, but after 36 days of starvation they were 37% of their original weight and only 22% the weight of controls (Fig. 1). There was no mortality in controls but starvation was continued until 50% of the slugs died. When fed again the starved animals grew three times faster than controls ($p < 0.001$) for about 20 days (Fig. 1). Following this their growth rate was similar to that of fed controls (Fig. 1). Despite compensatory growth, slugs that had been starved were only 38% the weight of fed controls after 100 days. There was remarkable variation in individual growth rates, and this was accentuated by starvation.

Some individuals lost weight slowly compared to others, and when re-fed some slugs grew slowly. Other individuals had compensatory growth rates much greater than the mean values illustrated in Fig. 1.

Adult *D. reticulatum* had negative growth rates but starved adults lost weight twice as rapidly as controls (Fig. 2). After 20 days fed and starved animals were 90.5% and 66% of their original weights, respectively. Although fed animals oviposited at a constant rate of about 1.2 eggs/slug/d over the 20 days, starved slugs produced 0.5 eggs/slug/day for the first 15 days and then produced none. Mortality increased steadily with time in both treatments. There was no mortality for the first 5 days, but after 20 days 80% of the starved slugs, and 60% of the fed animals had died. In a preliminary experiment with 82 adults collected in late fall, none withstood more than 27 days starvation.

Life history considerations

L. maximus showed a similar response to starvation as *D. reticulatum*. Slugs starved 288 h ate only 55% as much as those deprived 24 h (Table 3). This was a consequence of meal size since 91% of those starved 288 h ate a second meal compared to only 45% of those starved 24 h. The second meal was much smaller than the first. Thus, despite more frequent feeding, food consumption was reduced by longer starvation. The pattern of daily feeding was strongly bimodal. A large early peak of feeding occurred during the first 1.5 h of the dark period and a second, smaller peak occurred during h 3 to 4.5. Large amounts of pink saliva were secreted during feeding. One animal that weighed 5.4 g, for example, left 500 mg of saliva on the food.

DISCUSSION

D. reticulatum ate 2 to 4 meals per night but meal number varied strongly among individuals and diets. Meal frequency may be related to food hydration. There were at most, two meals of potato (hydration = 78%) (by *L. maximus*), three of carrot (hydration = 88%), and four of cucumber (hydration = 97%) (by *D. reticulatum*). In unpublished studies with *L. maximus,* cucumber was digested faster than carrot. Slugs ate more meals of moister foods and thus ate more on a wet-weight basis (Table 1) but greater amounts of drier foods were ingested on a dry-weight basis (0.583 of potato, 0.532 of carrot and 0.216 of cucumber (g dry food/g dry slug/day)). Rollo (1987) provides a more extensive analysis supporting this result. Despite differences in processing rates among diets, all meals were deposited as faecal strings within 24 h in agreement with observations by Pallant (1970) and Walker (1972).

Wet weight and volume were better comparative measures of meal size than dry weight (Table 1). Even small variations in the hydration of particular cucumbers produced noticeable differences among dry weight meal sizes (Table 1). Thus, although dry weight may be more important for productivity, wet weight or volume appear to be more important for regulation of ingestion. Volume was slightly more variable than wet weight, possibly due to compression of food in the crop, or water exchange between the food and body. Langer (1975) even suggested that instant mashed potatoes can kill slugs by swelling in their guts. Meal size is partially controlled by inhibitory feedback from the crop, presumably by stretch receptors (Susswein & Kupfermann, 1975a, 1975b; Senseman, 1978; Reingold & Gelperin, 1980), suggesting that volume should regulate intake. If these receptors were arranged to detect load, however, it could explain why wet weight was more consistent.

The behaviour of *D. reticulatum* suggested that compensation for starvation was occurring since the number and frequency of meals increased with increasing deprivation. Starved animals ate more meals and had shorter intervals between them. Due to decreased meal size, however, daily consumption was progressively reduced by increasing starvation (Table 1). The fact that starved animals fed more actively, and that their feeding the next day did not increase, suggests that a reduction in general metabolism cannot itself explain these results. Snails may arouse from dormancy in a matter of minutes and can rapidly alter their metabolic rate (Vorhaben *et al.,* 1984). Alternatively, slugs starved for long periods were slow to respond to the presence of food, and more of them did not feed which suggests that metabolism may have been depressed.

Slugs starved 100 and 220 h often remained in contact with the food long after eating whereas slugs starved less always returned to their resting sites. Food intake is regulated by

antagonism between signals that stimulate feeding (food palatability, empty crop, depleted reserves) and those that inhibit eating (feeding deterrents, adaptation of gustatory sense organs, full crop, high metabolic reserves) (Gelperin, 1971; Senseman, 1978; Reingold & Gelperin, 1980). Hunger may not be well represented by amounts consumed if reserves are important. For example, starved rats eat about the same sized meals as fed ones, but starved rats will ingest foods containing greater amounts of repellents (Miller, 1955). The extended association of starved slugs with their food may be related to continued demands from depleted reserves even after the crop is full. If so, it also means that sensory adaptation of the gustatory receptors takes considerably longer than the time required to feed, and so inhibitory feedback from the gut would be a more important regulatory mechanism.

Susswein & Kupfermann (1975b) found that starved sea slugs (*Aplysia*) ate most on the first day of re-feeding. Consumption on subsequent days was reduced by 37%–48%, similar to the results with *D. reticulatum* (Table 2). The reduced feeding in *Aplysia* was due to the presence of food in the anterior gut and the sole mechanism was inhibitory feedback from bulk. This feeding bottleneck may be exaggerated if passage of food through the gut is slowed to enhance assimilation efficiency (Calow, 1975b). There may be a switch to more rapid processing in the continued presence of food.

The fact that adult *L. maximus* did not exhibit compensatory feeding (Table 3), suggests that this response was not related to life history tactics (i.e., long-lived large adults versus short-lived small adults). Alternatively, immature *D. reticulatum* showed strong compensatory growth (and presumably feeding) following starvation (Fig. 1). Adults continued to reproduce and lost weight whether they were starved or not (Fig. 2). *L. maximus* also has a period of rapid growth during June and July, followed by reproduction and weight loss after mid-August (Rollo, 1983). Feeding was very high during the growth phase, but unexpectedly declined during reproduction (Rollo, 1983). Growth and reproduction in molluscs are interdependent and largely antagonistic. Hormones that stimulate maturation and reproduction simultaneously retard body growth (see Geraerts & Joosse, 1984). These results suggest that one phase of the life cycle is devoted to accumulation of resources (with compensatory control) and another phase is associated with output and lack of homeostasis.

It was surprising that adult slugs did not compensate for starvation, particularly since reproduction would presumably be enhanced by increased feeding (Sota, 1985). Cockroaches starved for two weeks had feeding rates four times greater than normal and elevated feeding persisted for more than two weeks (Rollo, 1984). Barton-Browne (1975) suggested that compensatory feeding following starvation might be universal in insects. What differences between insects and molluscs might explain these observations? One major difference is that insects store large quantities of lipid in localized sites (i.e. their fat bodies), whereas gastropods have relatively diffuse reserves of carbohydrates. Glycogen is stored in special connective tissue cells concentrated in the mantle, digestive gland and ovotestis of gastropods. The muscles also store glycogen and the albumen gland contains large quantities of galactogen (Veldhuijzen & Dogterom, 1975; Veldhuijzen & Cuperus, 1976; Widjenes & Runham, 1977; Hemminga *et al.,* 1985a, 1985b). Lipids act mainly as structural elements (1.36% of live weight in the terrestrial snail *Cepaea nemoralis*), not as energy stores (van der Horst, 1970; Horne, 1977). The glycogen reserves from various body compartments are mobilized during starvation to maintain concentrations of blood sugar (Veldhuijzen, 1975), but the galactogen from the albumen gland is not utilized (Veldhuijzen & van Beek, 1976).

A marked response of slugs to starvation was degrowth. After 40 d of starvation, immature *D. reticulatum* were 37% of their original body weight and they were only 22% the weight of controls (Fig. 1). Despite this, they were completely normal in appearance which is unlikely unless there was de-differentiation. Other soft-bodied invertebrates adjust their size to food conditions. Triclads, for example, may lose 90% to 97% of their body mass during starvation (Calow, 1977). Degrowth has been documented in aquatic snails in terms of protein loss (Russell-Hunter & Eversole, 1976) and de-differentiation (de Jong-Brink, 1973). A degrowth interpretation is also consistent with the results of Horne (1977) who showed that *Limax flavus* utilized protein as a major substrate during starvation. Degrowth cannot completely explain the lack of compensatory feeding following starvation in *D. reticulatum,* since feeding was reduced much more than

body size. After 220 h of starvation, feeding was only 57% of consumption by slugs starved 16 h whereas body size was 88.7% of original wet weight. Similarly *L. maximus* were 91% of their original wet weight after 288 h starvation but feeding was reduced to 55% of those starved 24 h (Table 3).

Some aquatic snails do show compensatory responses like those of insects. Calow (1975a) observed increased ingestion rates following starvation or on low quality food. Vianey-Liaud (1984) showed that immatures of the snails *Biomphalaria glabrata* and *B. pfeifferi* had compensatory growth rates following starvation. Like the present results with *D. reticulatum,* the compensatory period lasted about two weeks and starved snails never attained the size of fed controls. In another study (Hawryluk & Rollo, unpublished), we found that the aquatic snails *Stagnicola elodes* and *Physella gyrina* increased their daily consumption of food by 3.97 and 1.75 times, respectively, when their diets were diluted by 75% with cellulose. Reingold & Gelperin (1980) did not see such an increase with *L. maximus* when the diet was diluted with agar (but this also increased meal hardness) and no compensatory increase in feeding was observed in the sea hare, *Aplysia,* fed low quality food (Susswein & Kupfermann, 1975a, 1975b).

Insects, having extensive hard parts, may be better able to employ "set point" homeostatic control whereas slugs and snails may rely more on flexibility. Slugs in particular may scale their size in response to environmental constraints and opportunities. Thus, although molluscs are capable of long-term regulation of feeding (i.e., feeding responsive to reserve depletion or deviations from potential growth), degrowth may be more important, particularly after maturation and reallocation of reserves into reproduction. The ability of molluscs to rapidly alter their metabolic rate and degrow may make reliance on large reserves of concentrated energy (i.e., lipids) unnecessary.

More data are required before a model of feeding regulation can be constructed for gastropods. There appear to be major differences among species and with maturation. It would probably be worthwhile to examine changes in water content, and relative degrowth of particular organ systems during starvation. Changes in relative gut size might be especially important (see Sibly, 1981). Data addressing a greater number of species with divergent diets, morphologies and life histories are required.

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A QUANTITATIVE ANALYSIS OF FOOD CONSUMPTION FOR THE TERRESTRIAL MOLLUSCA: ALLOMETRY, FOOD HYDRATION AND TEMPERATURE

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ABSTRACT

Regression analysis was used to relate the dry-mass consumption of terrestrial slugs and snails to their body weight both intra- and interspecifically. The mean mass exponent for significant intraspecific analyses was 0.784 (7 species, 13 analyses). Multiple regression was performed for the interspecific analysis (686 cases, 18 gastropod species, 35 foods) so that variation associated with temperature and food hydration could be accounted for. The analysis explained 77% of the variation in the pooled data. Feeding was nearly in direct proportion to body weight (mass exponent of 0.919). There was a strong negative relationship between ingestion and the hydration of the food. The Q_{10} for consumption was 1.7. The general equation provided may be used as a standard for palatability studies.

Key words: allometry; feeding; terrestrial gastropods; temperature; palatability.

INTRODUCTION

There is a burgeoning literature concerned with application of allometric equations of the form $Y = aM^b$ that relate various physiological, behavioural and ecological attributes (Y) of organisms to their body mass (M) (reviewed by Peters, 1983; Calder, 1984; and Schmidt-Nielsen, 1984). The fact that so many aspects of organism design scale simply to an exponent of body mass (usually a power (b) of 0.75), suggests that there may be underlying rules or constraints that will allow us eventually to develop a unified theory of organism design.

Most allometric studies have dealt with vertebrates, and there are particularly few treatments of molluscs available. Von Bertalanffy (1951) included some data on the respiration of freshwater snails (*Planorbis* spp.), and Innes & Houlihan (1981) provided a more comprehensive investigation for respiration of intertidal gastropods. These appear to be the only studies addressing the Mollusca with any generality.

The present paper provides a general analysis of food consumption for the terrestrial Mollusca. Besides its theoretical interest, feeding by terrestrial gastropods is of major ecological and economic concern. These animals constitute an important component of most natural communities, acting as both primary herbivores and decomposers. Their impact on particular plant species (Pallant, 1969; Cates, 1975; Phillipson, 1983; Phillipson & Abel, 1983), and their role in nutrient cycling and energy flow requires knowledge of feeding rates (Mason, 1970a, 1970b; Pallant, 1974; Jennings & Barkham, 1975, 1976; Richter, 1979; Seifert & Shutov, 1981).

Terrestrial molluscs are also important pests of agriculture, sylviculture and floriculture (Runham & Hunter, 1970; Godan, 1983). Poisoned bait is the major method for slug and snail control (Wright & Williams, 1980). Consequently, knowledge of consumption is useful both for predicting the impact of slugs and snails on plant populations, and for projecting the effectiveness of control programmes.

METHODS

The daily food consumption of terrestrial molluscs was analysed with respect to their body mass, ambient environmental temperature and the water content of their food. The data consisted of 686 cases for 9 species of slugs and 9 species of snails. Suitable data (640 cases) were mainly obtained from published text and tables or were interpolated from figures (Mason, 1970a; Pallant, 1970; Stern, 1970; Gelperin, 1975; Jensen, 1975; Richardson, 1975; Williamson, 1975a, 1975b; Davidson, 1976; Jennings & Barkham, 1976; Richter, 1976, 1979; Williamson & Cameron,

1976; Morton, 1979; Senseman, 1978; Reingold & Gelperin, 1980; Wright & Williams, 1980; Bailey, 1981; Seifert & Shutov, 1981). These data were supplemented with 46 original observations. Additional information on temperature, hydration of foods, animal size and food consumption was obtained directly through correspondence with authors.

The amount of dry food consumed was used as the dependent variable. If authors reported wet consumption and did not provide food hydration, this was obtained from other studies or it was determined directly in the laboratory. Some types of leaves could not be obtained, and these cases were assigned a hydration of 82.08%, the mean hydration of other leaves (range, 75%–85%).

Dry tissue weight of the animals was used as an independent factor. Where authors used wet body weight and did not provide hydration, the value reported in other studies, or original observations were used. If no information was available it was assumed that the body was 89% water. This is typical of fully-hydrated terrestrial molluscs (Rollo *et al.*, 1983).

No correction was made for the small internal shells possessed by some species of slugs. However, only dry tissues were considered for snails. Where authors reported live snail weight, the percentage of the body mass attributed to the shell was obtained from the literature or original observations. Where no data were available, the animals were assumed to have shells similar to adult *Cepaea nemoralis* L. (i.e., shell = 15.46% of live weight).

In preliminary regression analyses using body weight as the independent variable, more of the variation in feeding was explained when the wet weight of the food was used instead of the dry weight. Consequently, the hydration of the food was included as an independent variable in the current analysis of dry weight consumption.

Temperature influences rates of physiological and behavioural processes and consequently is usually provided by authors. Where temperature was not reported it was sometimes obtained by correspondence or was assumed to be the same as in other studies conducted in the same laboratory. Where no information was available, a value of 15°C was assumed. The optimal temperature range for activity of most temperate terrestrial molluscs falls between 10°C and 20°C. Some species do not tolerate prolonged temperatures above 20°C, and so 15°C–18°C is the most common range selected by authors who are not specifically addressing the influence of temperature.

In addition to literature values, the analysis included original observations on daily feeding for the slugs *Deroceras reticulatum* (Müller), and *Limax maximus* L. These were obtained in the course of another study examining the regulation of intake of individual meals. *L. maximus* (n = 11) were fed cubes of potato tuber (*Solanum tuberosum* L.) in a light to dark cycle of 16:8 h at 18°C. *D. reticulatum* (n = 16) were fed cubes of carrot root (*Daucus carota* L.) or English cucumber (*Cucumis sativus* L., var. *anglicus* Bailey, n = 19). The animals were housed individually in jars with approximately 2 cm of moistened vermiculite on the bottom to maintain humidity (slugs do not ingest vermiculite). Slugs were maintained in the experimental conditions for several weeks prior to the observations.

The animals were starved for 24 h prior to each experiment to clear their guts of food and then they were weighed. Faecal strings were removed to prevent coprophagy. For each experiment, 20 cubes of the appropriate diet were weighed, dried to constant weight at 60°C, and re-weighed. This sample provided an estimate of the water content of the material. Each slug was given pre-weighed cubes of fresh food at the beginning of each dark cycle. The food remaining after 24 h was dried to constant weight at 60°C. The amount eaten was then calculated by subtracting the dry weight of the remainder from the estimated dry weight of the original food.

Intraspecific analyses were conducted using simple linear regression. For the interspecific analysis stepwise multiple regression and partial correlation analysis were performed using the Statistical Package for the Social Sciences (SPSS). In many instances authors reported the mean values of studies conducted with groups of animals instead of data for individuals. Clearly, a case based on 20 animals should have more weight than one based on a single specimen. Consequently, the analysis was performed with each case weighted (i.e., duplicated) for the number of animals that it represented. This resulted in the final analysis being based on 1,725 cases. The statistical model that accounted for the greatest amount of variation in the data was obtained by exploratory analysis (regressions and scatter diagrams) to find appropriate transformations to obtain linearity.

TABLE 1. Intraspecific regression analyses of food consumption (Log dry mg/d) for various gastropod species with respect to their body mass (Log dry mg shell-free tissue). Species marked with a "*" are freshwater and absorption rate was measured rather than consumption. Units for these species were μg/12 h for absorption and mg dry tissue × 100 for body mass.

Species	Constant (a)	Slope (b)	r^2	n	Probability	Temperature °C	Food	Author
*Ancylus fluviatilis**	1.26	.71	—	30	<.05	4.0	Algae	Calow, 1975
	1.89	.67	—	30	<.05	10.0		
	2.15	.70	—	30	<.05	18.0		
*Planorbis contortus**	1.20	.72	—	30	<.05	4.0	Detritus and Bacteria	Calow, 1975
	1.74	.71	—	30	<.05	10.0		
	2.80	.72	—	30	<.05	18.0		
Helix aspersa	.79	.349	.468	8	<.10	10.0	Lettuce	Mason, 1970a
	1.25	.331	.239	9	>.20	15.0		
Arion ater	−0.05	.514	.914	13	<.001	15.0	Lettuce	Stern, 1970
	6.27	−.029	.001	12	>.50	16.5	Agar diet	Jobin & Rollo, unpubl.
Ariolimax columbianus	1.49	.491	.849	33	<.001	15.5	*Oplopanax horridum*	Richter, 1976
	−2.78	1.002	.229	20	<.02	16.5	Agar diet	Jobin & Rollo, unpubl.
Deroceras reticulatum	2.24	.624	.189	19	<.10	5.0	Flour bait	Wright & Williams, 1980
	.48	1.394	.698	9	<.01	10.0		
	.32	.464	.099	22	>.10	18.0	*Ranunculus repens*	Pallant, 1970
	−.76	.759	.493	19	<.001	18.0	Cucumber	This study
	2.67	.182	.027	16	>.50	18.0	Carrot	This study
Deroceras laeve	−.32	.619	.864	26	<.001	16.5	Agar diet	Jobin & Rollo, unpubl.
Cepaea nemoralis	3.26	−.053	.011	19	>.50	20.0	Lettuce	Richardson, 1975
Milax budapestensis	.12	1.183	.247	27	<.01	15.0	Flour bait	Wright & Williams, 1980
	3.65	−.069	.001	17	>.50	5.0		
Limax maximus	10.33	−.617	.107	11	>.20	18.0	Potato	This study,
	3.39	.185	.078	16	>.20	16.5	Agar diet	Jobin & Rollo, unpubl.
Lehmannia marginata	1.37	.113	.057	23	>.20	16.5	Agar diet	Jobin & Rollo, unpubl.

RESULTS

Table 1 is a compilation of intraspecific allometric relationships for consumption and body mass for terrestrial molluscs for which there were enough observations. Some data on absorption rate from aquatic species are also provided. There was considerable variation among studies with respect to the mass exponent (range of −0.617 to 1.394). The mean (0.486), was exceptionally low. If only those values that were significant ($p < 0.05$) are considered, however, the mean value was 0.784.

The best interspecific analysis using stepwise multiple regression is presented in Table 2. This was obtained when both the dry body mass and dry daily consumption were converted to natural logarithms. A combination of log body mass, temperature and hydration resulted in a highly significant regression with $r^2 = 0.7742$. This means that 77%

TABLE 2. Multiple regression analysis of food consumption (Log dry mg d) of terrestrial Mollusca with respect to their body mass, environmental temperature and food hydration. Bracketed values resulted when the weighting procedure was not employed (see text). $r^2 = 0.77417$, n = 1,724, p · . 0.00001; ($r^2 = 0.67287$, n = 685, $p < 0.0001$)

Variable	B (Slope)	Standard error	Probability
Body mass (Log dry mg)	0.9191051 (0.8593249)	0.0126404 (0.0250956)	< 0.00001 (< 0.0001)
Temperature (°C)	0.0539130 (0.0353888)	0.0050549 (0.0100655)	< 0.0001 (< 0.001)
Food hydration (%)	−0.0374423 (−0.0385831)	0.0011767 (0.0016202)	< 0.0001 (< 0.00001)
Constant	0.2374659 (0.8768701)	0.1139649 (0.1700361)	< 0.037 (< 0.0001)

of the observed variation in daily consumption was accounted for by the regression with these three variables (Zar, 1974). Interaction effects were also examined (e.g., temperature × body mass), but none were significant.

Use of the weighting procedure resulted in statistical calculations based on a larger sample than was originally available. For those readers who may hesitate to accept the calculated probabilities in this case, the values obtained when no weighting was employed are also presented (Table 2). The weighted coefficients and constant are certainly the most appropriate. The coefficients calculated without weighting were slightly different and so they are also provided in Table 2.

Because consumption is influenced by all three independent factors, as well as other variables, the trend associated with a single factor is not always readily apparent unless it accounts for a large amount of the variation. Partial correlation analysis was performed to examine the association of consumption with particular variables while controlling for the influence of the others. When temperature and food hydration were controlled, body mass accounted for 75% of the residual variation in feeding. When body mass and temperature were controlled, food hydration accounted for 37% of the remaining variation. When body mass and food hydration were controlled, temperature accounted for 6% of the residual variability.

It is possible to illustrate these relationships by computing the appropriate residuals from the raw data. The variation associated with a particular factor can be removed by appropriate transformation of the dependent variable. For example, if feeding were directly proportional to body mass, and the influence of hydration was to be examined, the influence of weight could be removed by simply dividing consumption by the animal's body mass to obtain an independent variable with the units mg eaten/mg body mass. This is common practice. Since the present analysis found that feeding was proportional to an exponent of body weight, the coefficient derived from the multiple regression analysis was employed to this effect (Table 2). A mass exponent of 0.75 is sometimes employed in the literature in this manner (e.g., Sibly, 1981). Thus the transformation to remove the variation associated with body weight is:

$$YT = YO - 0.91910509(X)$$

where YO = log dry consumption (mg), X = log dry body mass (mg), and the coefficient was obtained from Table 2.

Similar transformations can be employed to remove the variation associated with temperature or hydration of the food. The effect of such transformation is illustrated in Figs. 1 and 2. Fig. 1 shows the relationship between dry consumption and dry body weight using simple linear regression with logarithmic transformations of both variables. Many of the high values of consumption for smaller gastropods were associated with baits that had low water content. Temperature effects also obscure the relationship. Fig. 2 illustrates the relationship between consumption and body mass when the dependent factor has been transformed to

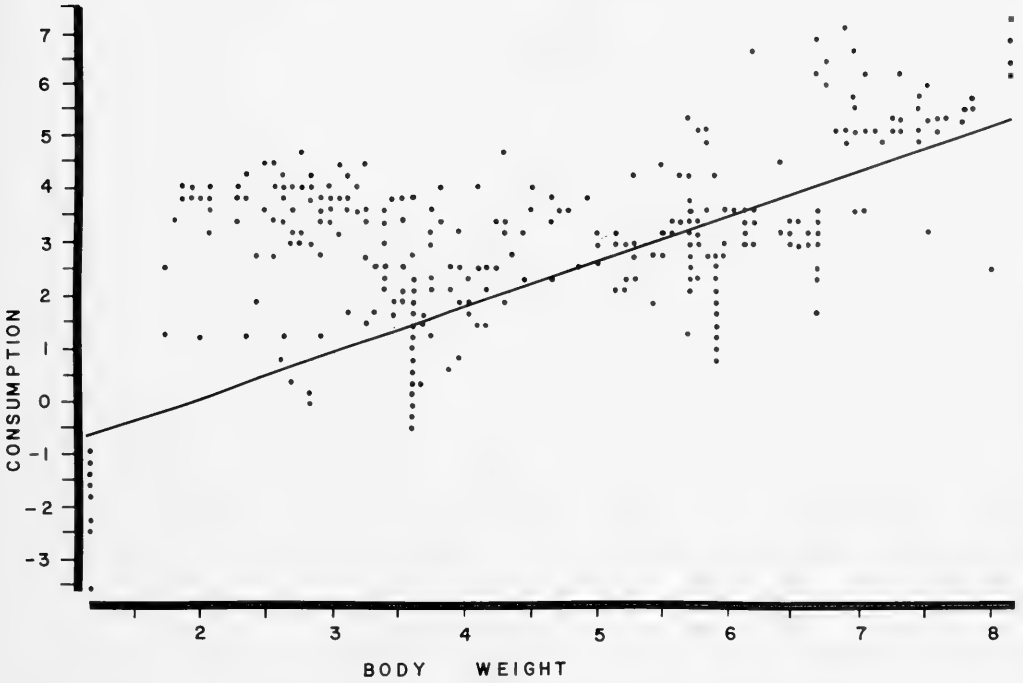

FIG. 1. The relationship between food consumption by terrestrial gastropods and their body weight. Simple linear regression of (C) consumption (log dry mg) with (W) body weight (log dry mg). The equation for the best fitting line was: $C = 0.87409(W) - 1.75540$; $r^2 = 0.64000$, $p < 0.00001$, $n = 1{,}725$.

remove the variation associated with both temperature and food hydration.

It was very difficult to discern the relationship between consumption and temperature or between consumption and food hydration in the original data because of the large influence of weight and other factors. The trends are apparent, however, following appropriate transformations of the dependent variable. Fig. 3 illustrates the relationship between food consumption and temperature when variation due to body weight and hydration have been removed. Similarly, Fig. 4 illustrates the relationship between consumption and food hydration when the variation associated with body weight and temperature have been removed.

The relationship of consumption to temperature appears linear over the range of temperature examined (Fig. 3). Given the coefficient for temperature from the multiple regression analysis (Table 2), it is possible to obtain the Q_{10} for consumption using the relationship:

$$\text{Log}(Q_{10}) = 10(B)$$

where B = the coefficient for temperature from Table 1 (Innes & Houlihan, 1981). This was checked by using the standard equation for calculating Q_{10}:

$$\text{Log}(Q_{10}) = (\text{logRate 2} - \text{logRate 1})\ (10/23.5–5.0)$$

where the rates were calculated by substituting the mean animal mass and food hydration into the multiple regression equation (Table 2).

The calculated Q_{10} for consumption of terrestrial Mollusca over a temperature range of 5°C to 23.5°C was 1.7145 using either formula.

DISCUSSION

The intraspecific mass exponent for metabolism generally has a value of 0.66 (Heusner, 1982; Feldman & McMahon, 1983; Wieser, 1984). Table 1 showed high variation in the value of b for consumption, with an exceptionally low mean of 0.486. Very few of these

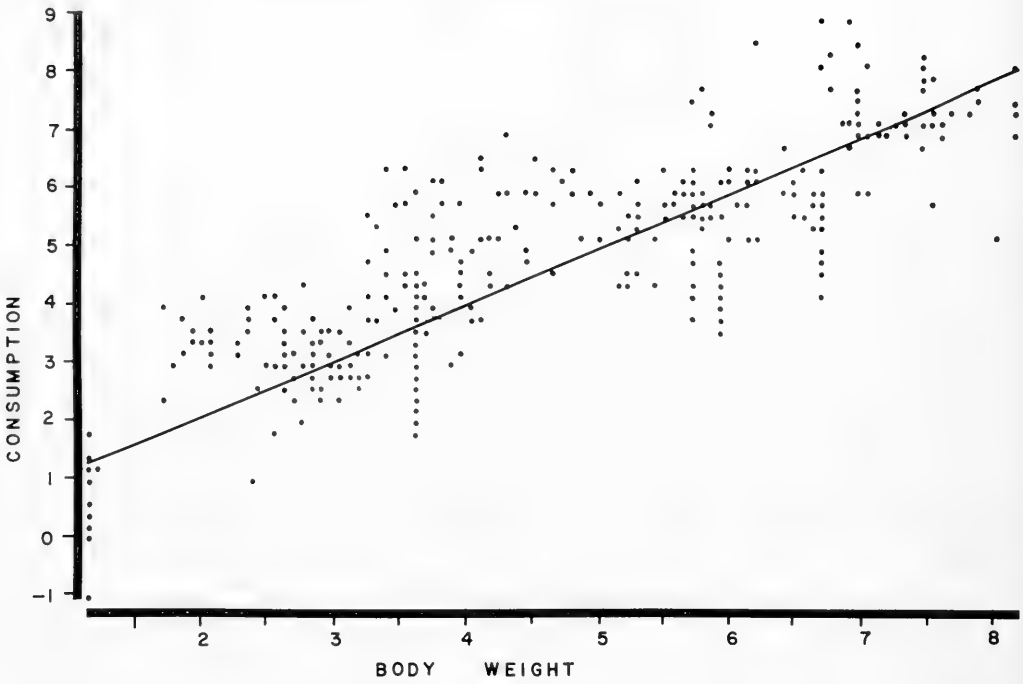

FIG. 2. Simple linear regression of consumption with body weight when the dependent factor (CT) was transformed to remove variation associated with (T) temperature (°C) and (H) hydration of the food (%). The transformation was: CT = C − 0.053913044(T) + 0.037442321(H). The equation for the best fitting line was: CT = 0.91911(W) + 0.23747; r^2 = 0.75807, $p < 0.00001$, n = 1,725. Repeated values are not indicated in the figures.

studies were conducted with deriving allometric relationships in mind. Consequently, the animals were often matched for size or developmental status. In addition, feeding may be relatively high in young growing stages but may decline in adult or senescent individuals (Wieser, 1984). The mass exponent will be strongly affected by the range of body sizes used and the age structure of the sample. This probably explains much of the variation in the calculated values (Table 1). If only studies which were significant are considered, the mean mass exponent was 0.784, close to the value predicted for interspecific comparisons. Further data will be required to determine if terrestrial molluscs in fact obey the surface rule (i.e., b = 0.66) intraspecifically.

Heusner (1982) showed that the value of the mass coefficient for metabolism (a) increases with body mass in mammals. This indicates that metabolic power or basal metabolism increases in larger species (Wieser, 1984). There was no clear trend between the mass coefficients for feeding and body mass in the present analysis (Table 1), although such a trend might emerge in a more controlled comparison. Calow's (1975) data show a clear influence of temperature on the mass coefficient but they also suggest that the mass exponent is not affected by temperature.

For the interspecific analysis, the regression model that best described the data conformed to the standard allometric relationship found to apply in studies of other animals, except for the addition of further variables (see Peters, 1983). Considering that the raw data are based on 18 species feeding on 35 different diets, it is remarkable that body weight, temperature and food hydration account for 77% of the observed variation (Table 2). This leaves only 23% of the variation to be accounted for by species differences, individual variation, acclimation, age, maturation state, and activity levels of the gastropods or the palatability, physical properties, nutritional value and energy content of the

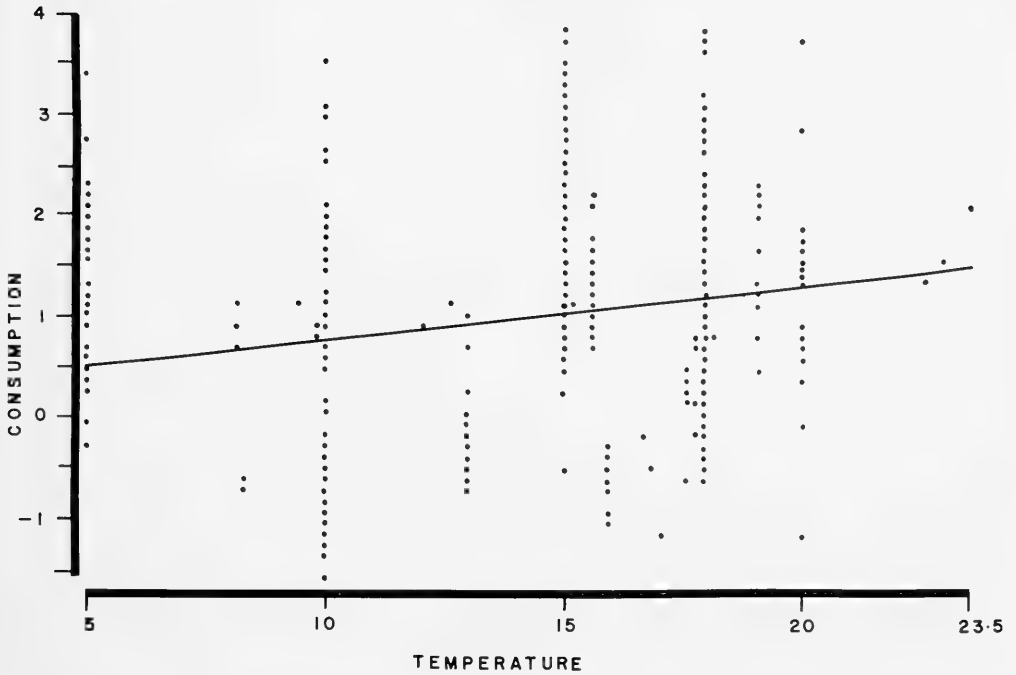

FIG. 3. The relationship between food consumption by terrestrial gastropods and (T) temperature (°C) (log dry mg) was transformed to remove the variation associated with (W) body weight (log dry mg) and (H) food hydration (%). The transformation was: $CT = C - 0.91910509(W) + 0.037442321(H)$. The equation for the best fitting line was: $CT = 0.05391(T) + 0.23747$; $r^2 = 0.06584$, $p < 0.00001$, $n = 1{,}725$.

food. This is all the more remarkable since errors arising from assumptions concerning incomplete data, linearity of the relationships, and errors associated with measurement must introduce considerable variation in this kind of analysis.

Von Bertalanffy (1951) identified three classes of animals: those whose respiration was directly proportional to their body weight, those whose respiration was proportional to their surface area, and those whose respiration was intermediate between these extremes (i.e., mass exponents of 1.00, 0.66 and 0.75 respectively). For aquatic snails he found a mass exponent of 0.75. Subsequent research across wide phylogenetic boundaries has shown that most behavioural, physiological and ecological features scale to this mass exponent interspecifically (Peters, 1983; Calder, 1984; Schmidt-Nielsen, 1984). Innes & Houlihan (1981) found a mass exponent of 0.724 for the respiration of intertidal gastropods in air, and 0.701 for those in water (range in equations, 0.63–0.81). This is consistent with Von Bertalanffy's (1951) results.

Von Bertalanffy (1951) also observed, however, that terrestrial snails of the family Helicidae exhibited respiration rates directly proportional to their body weight. The present results show that daily feeding of terrestrial molluscs also has a mass exponent (0.91) much closer to direct proportionality than to 0.75 (see Figs. 1 and 2). In most other organisms examined, ingestion has a mass exponent close to 0.75 (Peters, 1983). There is no accepted explanation of why particular organisms exhibit a particular mass exponent. If the apparent difference between aquatic and terrestrial molluscs is real, however, an answer may emerge from comparative studies.

One problem with the current data is that various species are not equally represented and there is probably a bias towards adult animals. This could influence the mass exponent and may explain the difference between exponents when weighting was applied or not (Table 2). What is required is a systematic survey in which a range of species is compared across developmental stages and un-

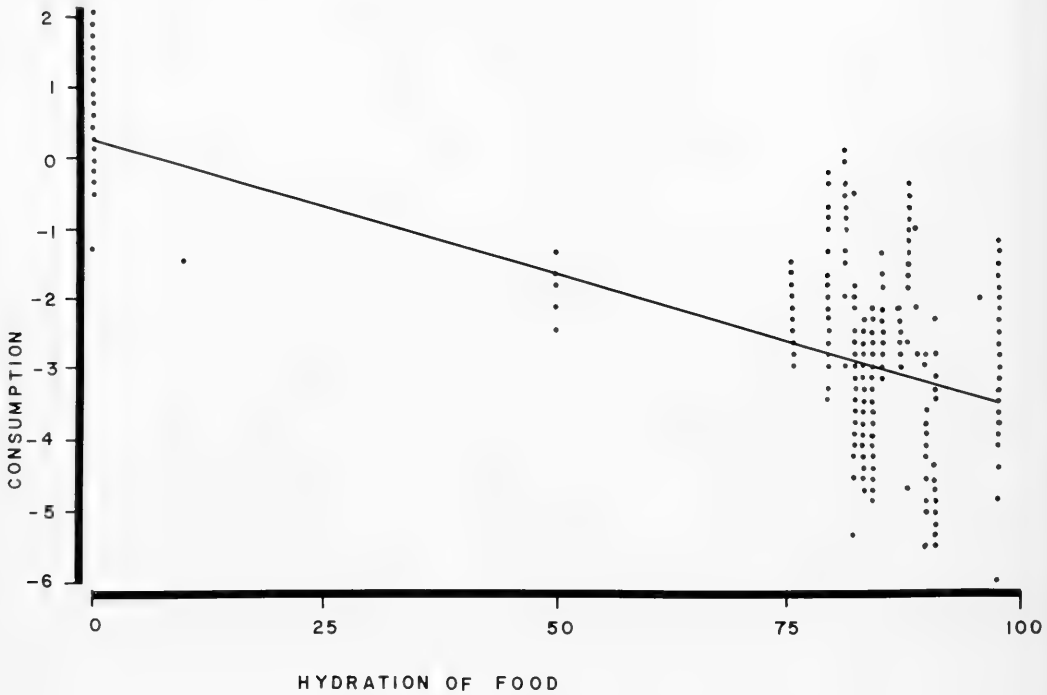

FIG. 4. The relationship between food consumption by terrestrial gastropods and (H) food hydration (%). Consumption (C) (log dry mg) was transformed to remove variation associated with (W) body weight (log dry mg) and (T) temperature (°C). The transformation was: $CT = C - 0.91910509(W) + 0.053913044(T)$. The equation for the best fitting regression line was: $CT = 0.23747 - 0.03744(H)$; $r^2 = 0.38847$, $p < 0.00001$, $n = 1{,}725$. Repeated values are not indicated in the figures.

der standard environmental and nutritive conditions. We are currently carrying out such a project using an artificial diet embedded in agar. A preliminary analysis using 5 species of slugs reared in identical conditions provided an interspecific mass exponent of 0.697 (Jobin and Rollo, unpublished). Nevertheless, the present analysis indicates that there is a very clear trend in the interspecific data and the line with a slope of 0.919 appears to describe it very well (Fig. 2). The range of species of vastly different sizes and the large number of observations should provide a fairly accurate assessment of interspecific trends. In addition, this analysis allows the variation associated with temperature and food hydration to be addressed.

The interspecific Q_{10} for consumption of terrestrial gastropods was 1.715. This is very close to the values obtained for intraspecific absorption rate in freshwater snails by Calow (1975) (range of 1.38 to 1.82). Terrestrial molluscs are generally adapted to relatively cool temperatures which are in the range of experimental temperatures encountered (5°C–24°C). Even with size and food hydration controlled, however, temperature only accounted for about 7% of the residual variation (Fig. 3). Although most species show maximum activity between 15°C and 20°C, there are differences among species. For example, gastropods such as *D. reticulatum, Deroceras laeve* (Müller) and *Arion hortensis* Férussac remain active at lower temperatures than species such as *L. maximus* or *C. nemoralis.* Thus different species have different preferred temperature ranges and this may obscure the influence of temperature in the general analysis.

In addition, the relationship between temperature and behavioural or physiological processes is not linear within a species. Typically, rates are zero at lower and upper temperature thresholds. Within this range processes tend to accelerate gradually with increasing temperatures to an optimum, following which they decline precipitously (e.g., Rollo, 1982). Acclimation can shift this tem-

perature response curve (Rising & Armitage, 1969), and this is probably an additional source of variation.

The data show a simple linear increase with temperature (Fig. 3), instead of the parabolic response curve that would be expected for a single species. Zero values would undoubtedly be obtained, however, if observations at even higher and lower temperatures were obtained. Although the pooled data show the general temperature response of terrestrial gastropods with respect to feeding, they may overestimate the temperature range for any single species.

Moisture is a major limiting factor for terrestrial molluscs because they have unrestricted evaporation from their epidermis and must secrete a ribbon of mucus to move (Machin, 1975). It might be expected that foods with higher water content would be preferred. A large component of most diets is water, however, and this can contribute substantially to the volume of a meal. Since feedback from stretch receptors in the crop is a major factor controlling meal size (Senseman, 1978; Reingold & Gelperin, 1980), the dry weight of material ingested may be physically limited where there is a large structural component of water. Meal intake is also influenced by the concentration of gustatory stimulants in the food (Senseman, 1978; Reingold & Gelperin, 1980), and this would be higher in drier diets. This combination of effects probably explains the strong negative relationship between daily feeding and the hydration of the food (Fig. 4).

Most of the studies considered for the present analysis ensured that the animals were fully hydrated. The ability of slugs and snails to become active and forage is influenced by their hydration (Rollo *et al.,* 1983). Thus, dehydrated animals may exhibit behavioural changes in preference associated with the water content of their food. They may also be physically limited by their inability to prevent losses of body water to osmotically concentrated food in the digestive tract. Quantitative studies are needed in this area.

Although there is a large literature concerned with feeding by terrestrial molluscs, probably less than 10% of it was sufficiently complete and quantitative, or presented in a form that allowed an analytical synthesis. There are a number of key problems limiting the generality of the literature that can be corrected. For example, the most common measurements of food consumption are either surface area removed (Judge, 1972; Cates, 1975; Jennings & Barkham, 1975; Reader & Southwood, 1981; Rathcke, 1985), weight (wet or dry) or energy. Area consumed may be a valuable measurement if the impact of the gastropod on a plant's photosynthetic capacity is of interest. Due to variation in leaf thickness, consistency and morphology, however, a given amount of area removed may represent a different amount of absolute consumption depending on the plant species considered. Authors should provide a surface-area-to-weight conversion factor for the material they are using. Similarly, if energetic units are employed, an energy-to-mass conversion factor should be provided. The water content of the diet is also an important factor (Table 2, Fig. 4).

When the size of snails is given, various linear measurements of shell morphology are often used. This may be an advantage when the animals must be left alive since weight may vary with consumption and hydration. Shell morphology and thickness varies with species, age and environment, however, and authors often measure different aspects (e.g., aperture width or shell length). For generality, authors should provide the relationship between the measures employed and the weight of dry tissues.

A major focus of feeding studies is the palatability of gastropod foods. Molluscs have been used as model "general herbivores" to test foraging hypotheses (Cates & Orians, 1975; Rathcke, 1985). The use of palatability or acceptability indices to rank the relative attractiveness of diets is standard practice. These indices are usually calculated by comparing the amount of a particular food eaten to a standard that is highly preferred by a gastropod. Because the resulting index is a relative measure it has no generality, especially since different studies use different standards (see Grime *et al.,* 1968, 1970; Cates & Orians, 1975; Richter, 1976; Dirzo, 1980; Richardson & Whittaker, 1982; Whelan, 1982; Rathcke, 1985). Furthermore, the ranking of the diet may change according to which plant is used as the reference (even within a single experiment), and depending on whether dry weight or leaf area consumed is measured (Richardson & Whittaker, 1982). These problems of standardization and generality may be overcome if authors use the statistical model developed here as a reference. The degree of deviation from the predicted consumption (positive or negative) could serve as a measure of preference and

would ensure that all studies use the same units and consider all the relevant factors.

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MATING BEHAVIOUR OF *LYMNAEA STAGNALIS*

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ABSTRACT

Mating behaviour of the pond snail *Lymnaea stagnalis* was analysed in pairs of snails, reunited after a period of isolation. Apparent female behaviour is absent in this situation, whereas male behaviour is characterized by a series of consecutive acts: mounting, turning, eversion of preputium, intromission, withdrawal of preputium and moving off. Several variations on this basic pattern occur, of which sham-copulation (false coupling) and reciprocation (reversal of roles after completion of copulation) are the most remarkable ones. The same behavioural sequence appeared to be present in spontaneous matings. Prior experience is not needed for the performance of mating behaviour. The duration of the behaviour is variable. This is mainly due to the latency of intromission. The duration of intromission is fairly constant (36 ± 4 min). Inspection of the vagina of female copulants for the presence of semen revealed that 90–100% of the copulations is successful. Surgical removal of the part of the vas deferens that runs through the body wall eliminates all male copulation behaviour without affecting the ability of copulation as a female.

Key words: *Lymnaea stagnalis,* mating, male and female behaviour.

INTRODUCTION

The reproductive biology of the simultaneous hermaphrodite freshwater snail *Lymnaea stagnalis* (Linnaeus) has received much attention in recent years. The first systematic study of the relation between copulation and egg laying was conducted by Horstmann (1955). Since then, laboratory studies have been done on endocrine and neurophysiological control of ovulation and oviposition, the structure and maturation of the reproductive system, external factors affecting fecundity and related issues (for reviews see Joosse & Geraerts 1983, Geraerts & Joosse 1984). An experimental study on the fecundity of *L. stagnalis* in the field was presented by Brown (1979).

Mating in freshwater pulmonate snails—mostly simultaneous hermaphrodites—is not a necessary condition for egg-laying in many species: under conditions of isolation they may reproduce by internally self-fertilized eggs (Duncan, 1975, Geraerts & Joosse, 1984). Yet mating must be considered a major element in the reproduction tactics of these hermaphrodite animals for several reasons: (1) Studies using genetic markers demonstrated that after the snails have been allowed to mate, cross-fertilized offspring is produced for some weeks by animals that have copulated as females (*Biomphalaria glabrata:* Paraense, 1956, 1959, Richards, 1970; *L. stagnalis:* Cain, 1956). (2) In *L. stagnalis,* isolated before the appearance of sexual behaviour, the onset of egg-laying is delayed by two or more weeks due to the absence of foreign semen (Van Duivenboden, 1983). (3) Once egg-laying has started, mating reduces fecundity in those lymnaeids studied (De Witt & Sloan, 1958; Van Duivenboden *et al.,* 1985).

Under laboratory conditions *L. stagnalis* commences mating activity at the age of 7–8 weeks (shell height about 18 mm). Egg-laying starts 2–3 weeks later. Once egg-laying has started, the snails show continuous mating and oviposition activity.

A general description of the copulation behaviour of *L. stagnalis* was given by Noland & Carriker (1946) and a more detailed one by Barraud (1957). The latter concluded that copulation was seldom successful. This seems to contradict the studies mentioned above, because it implies that the complex and time-consuming mating behaviour hardly

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serves any purpose in reproduction. Moreover, Barraud suggested that a systematic study of mating in this snail is hardly possible.

In recent years we studied masculinity and receptivity in *L. stagnalis* (Van Duivenboden & Ter Maat, 1985) and effects of mating on egg-laying (Van Duivenboden, 1983, Van Duivenboden *et al.*, 1985). From these studies a detailed description of the mating behaviour of *L. stagnalis* was compiled, which is presented here.

Copulation behaviour can be induced readily by reunion of snails after a period of isolation (Noland & Carriker, 1946, Rudolph, 1979a). This method was used to describe mating behaviour and the results were compared with spontaneous matings as well. The success of copulation was assessed through dissection of female copulants, immediately after mating. Finally a simple operation was performed, which completely eliminates male mating activity.

METHODS

Laboratory-bred specimens of *L. stagnalis* were used. They were raised and kept in tanks with continuous water change at a temperature of 20 ± 1°C (Van der Steen *et al.*, 1969). A 12/12 light/dark cycle was maintained with overhead fluorescent lighting.

Isolation was performed by placing the animals individually in perforated polyethene jars in the tank. They were fed lettuce leaves *ad libitum* (cf. Scheerboom, 1978). After six or more days of isolation, mating was induced by housing the animals in pairs in clean jars (one pair per jar) filled with 250 ml of fresh aerated tap water in a temperature controlled room (20°C). The snails were marked with nail polish at the tip of the shells to simplify identification during observation. At first the behaviour was observed continuously to develop criteria for the analysis of behaviour. Mating behaviour in snails is very slow so two persons can observe adequately 20–25 pairs at a time by brief observations at 1 min intervals.

Spontaneous mating behaviour was observed in the 800 liter breeding tanks (with up to 700 snails per tank) in the laboratory. The animals were fed three times a week, alternatively lettuce leaves and fish food (Tetraphyll, Tetrawerke A.G.), in restricted amounts.

Female copulants were dissected immediately after copulation. The vagina normally looks flaccid and transparant and it is difficult to recognize. A copulation was considered as successful when the vagina had a white, swollen appearance (3–5 times its normal size). In those cases the otherwise transparant duct of the bursa copulatrix was also filled with white material.

The animals were anesthetized with $MgCl_2$ (Van Duivenboden, 1982). A small cut was made in the body wall to remove some mm of the vas deferens. The vas deferens was interrupted in either one of two places: 1) where it runs freely in the rear sinus, or 2) the part that runs through the body wall (see Fig. 5 for anatomical details). Sham-operated animals were treated like the operated ones, except for the cutting of the vas deferens. Recovery required up to 4 hours.

Frequency data were tested by means of the G-test after Williams' correction. Analyses were carried out according to Sokal & Rohlf (1981). Data were tested for normality with the method of Shapiro & Wilk (1965, 1968) and for homogeneity of variances with the F-max procedure.

The terms "male" and "female" refer to the male and female copulant, respectively.

RESULTS

Isolation-induced mating

Mating behaviour of about 750 pairs of snails was observed, in a series of fifty experiments. After an isolation period of six days or longer, 75–100% of the pairs showed mating behaviour.

Male mating behaviour stood out clearly from all other behaviours exhibited by *L. stagnalis*. A number of consecutive behavioural acts could be distinguished in all successful copulations in males, but not in females. Females seemed to behave indifferently for the greater part of the time, moving about, air breathing and feeding during all phases of copulation. Although we will therefore focus on the male, some reactions of the female will be discussed. Firstly, a description of a straightforward mating sequence, will be given. This will serve as the basic framework for the treatment of all other behaviour accompanying mating.

When the isolated snails are paired, they crawl about in a seemingly random fashion. When they meet, the prospective male

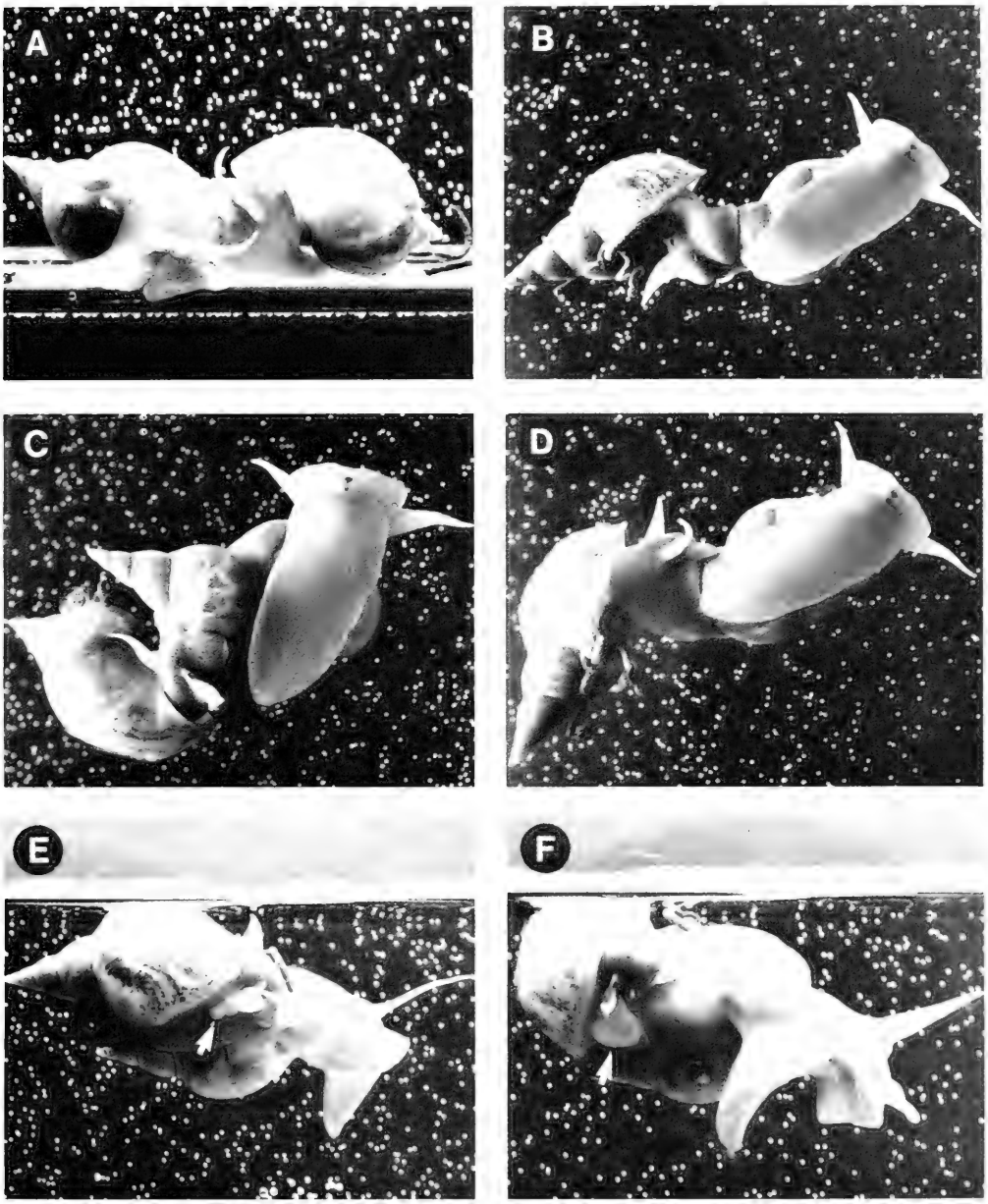

FIG. 1.A. Mounting, male at right. B, C, D. Turning, male at left. E, F. Partial eversion of preputium (arrow), male at left.

mounts the prospective female (mounting) and starts rounding the apex of her shell (turning), always in a (characteristic) counter-clockwise manner (seen from above). The male opening, near the base of the right tentacle, becomes visible as a white dot, indicating that the eversion of the preputium had started (partial eversion of preputium) (Fig. 1A-F). When the male reaches the aperture of the shell of its partner—at the right hand side—the male comes to an almost complete stop. At this point the head/foot part shortens and gets a swollen appearance. The tentacles are shortened and drooped and the

apex of the shell is kept down. This posture is specific for mating snails. Progress is now very slow, as the right side of the foot of the male travels along the margin of the shell of the female. Meanwhile swellings and contractions occur in the partially everted preputium. Locomotion ceases altogether when the lips and tentacles of the male have passed the pneumostome of the partner. The preputium is then totally everted and the tip makes searching movements under the female's shell. A totally everted preputium is unmistakable because of its large size (length 10–20 mm, width 5–10 mm in adult snails). The actual eversion of the penis can only occasionally be observed. However, once the female gonopore of the female copulant is occupied by the tip of the preputium of the male, intromission is highly probable (see below: semen transfer). During intromission, undulations of the vas deferens are visible through the transparant wall of the preputium. The preputium is subsequently withdrawn and the male moves away. During mating the male is very firmly attached to the shell of the female: copulating animals can only forcibly be separated.

Fig. 2 summarizes the mating behavior. Thick lines in the diagram indicate the basic features of the behavioural sequence as described above. Thin lines refer to events which may complicate mating. Several mountings, whether or not followed by turning and rarely even by partial eversion of the preputium, may occur, after which the animals separate and start again. The snail which was the first to mount will generally, but not always, become the male. Occasionally reversal of roles occurs during the mounting or turning phase. During the phase of partial eversion of preputium, role reversal is very rare and during the phase of total eversion of the preputium role reversal never occurs.

Another complication occurs when the preputium is totally everted before the male is in the right position. Two things may happen then: (1) the preputium makes some searching movements under the female's shell, is then partially withdrawn, and the male makes one or more turns followed by a second attempt, or (2) a "sham"-copulation takes place, i.e. the preputium is put under the shell of the female without subsequent intromission and ejaculation.

A sham-copulation is generally characterized by strong withdrawal of the forepart of the female, after which she relaxes again and may resume locomotion or floating, while the preputium remains in place (Fig. 3A–C). This situation continues for 15–60 min, or even for some hours. In most sham-copulations the preputium is placed between the tentacles of the female (frontally) but every position at the margin of the shell is possible. The preputium may even be inserted in the pneumostome of the partner. Sham-copulation comes to an end by partial withdrawal of the preputium. One or more turns are then made, followed by a second attempt. When the male was already in the correct position, the turns may be omitted. Up to two successive sham-copulations may precede intromission. Sham-copulations occur frequently (≥50% of the pairs, see Table 1).

TABLE 1. Latency from pairing and duration of intromission and the occurrence of sham-copulation in isolation-induced matings. A.: Snails with shell heights of 31–35 mm, aged 13–17 weeks. B: Snails with shell heights of 21–24 mm, aged 9 weeks.

Period of isolation (days)	N	Intromission: Latency Mean ± SD (min)	Intromission: Latency CV[a]	Intromission: Duration Mean ± SD (min)	Intromission: Duration CV[a]	Sham-copulation
A. 6	20	158 ± 46	0.29	36.4 ± 3.2	0.09	50%
8	5	143 ± 68	0.47	34.6 ± 2.7	0.08	80%
11	16	135 ± 51	0.38	36.5 ± 4.1	0.11	94%
16	13	89 ± 31	0.35	36.2 ± 3.2	0.09	77%
B. 16[b]	31	95 ± 29	0.30	35.9 ± 3.7	0.10	62%

[a]CV = Coefficient of Variation.
[b]Isolated before the appearance of sexual activity.

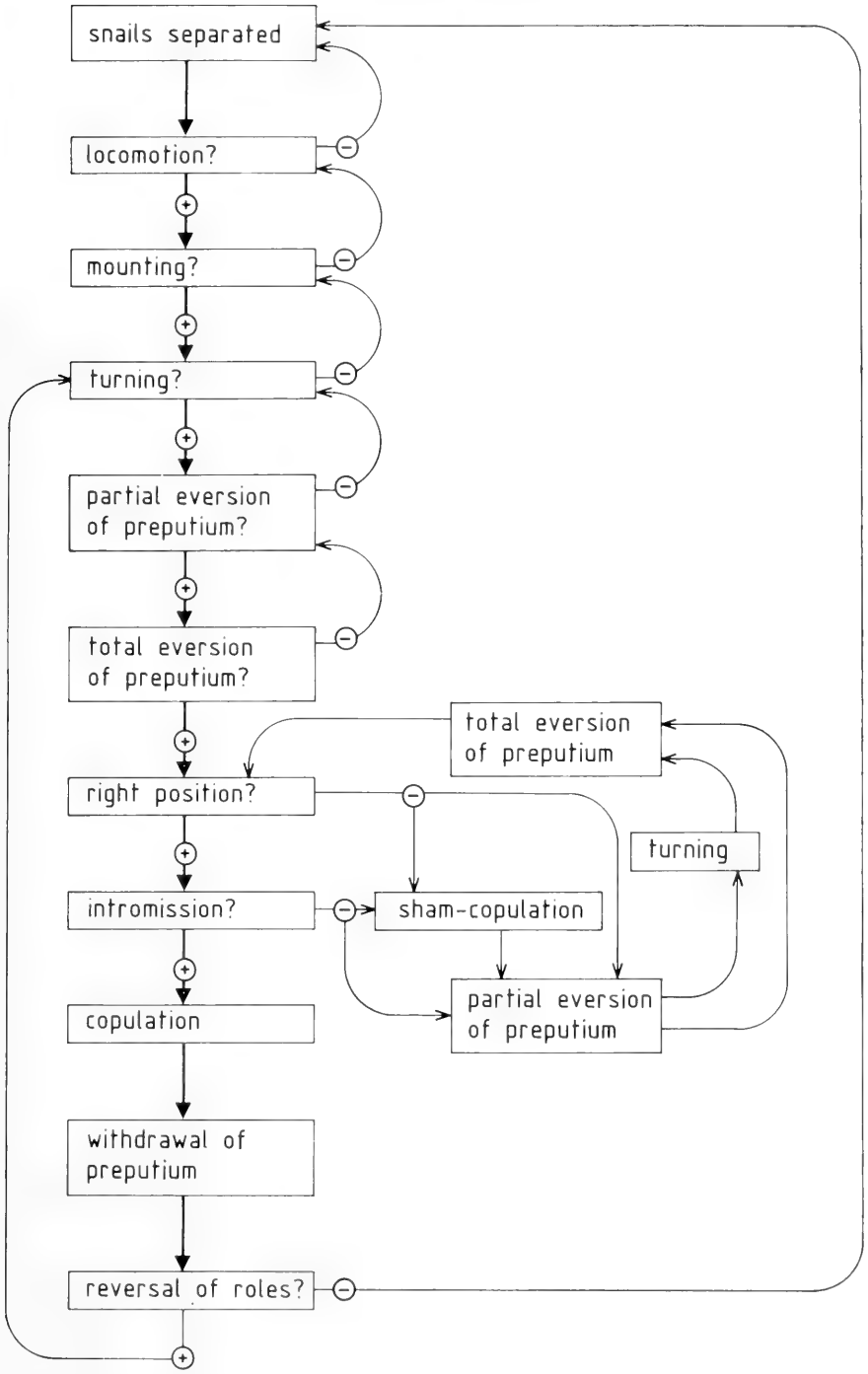

FIG. 2. Diagram of the mating behaviour of *L. stagnalis*. Thick lines refer to the basic features of male mating behaviour. Thin lines refer to behaviours which often accompany mating. See text for details.

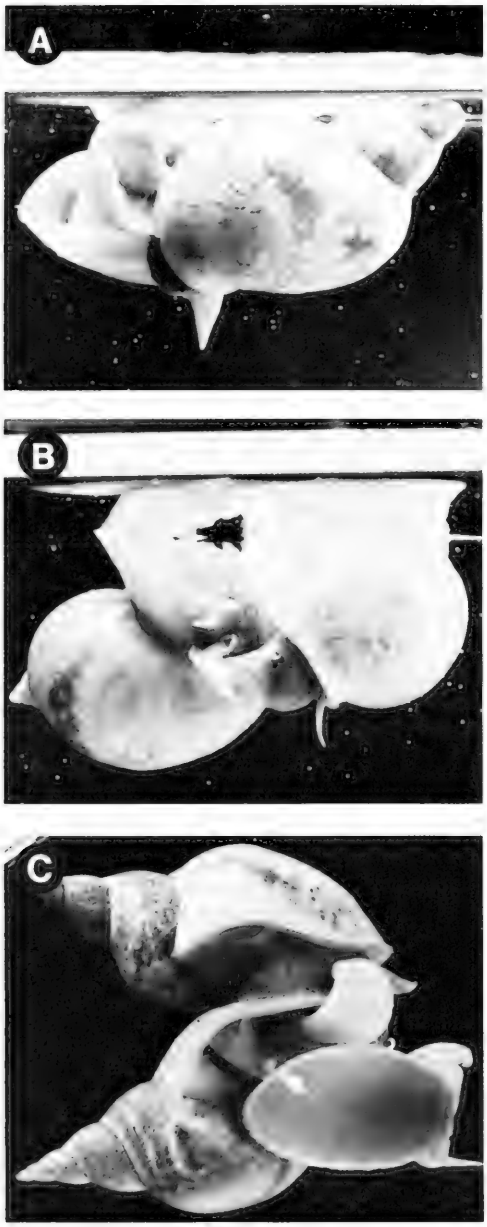

FIG. 3. A. Contorted retraction of the female (at left). B. Relaxation of the female (at left). C. Fully relaxed female resuming locomotion (lower snail), carrying the male upon her shell. Arrow: unoccupied female gonopore of the female, indicating sham-copulation.

During intromission the female may mount the shell of the male, while *in copulo*. This results in an extremely complex posture of both snails (Fig. 4A–E). After intromission is completed in this situation, the male loses foothold and a second mating sequence with the roles reversed takes place. Reciprocal copulation contains all the basic behaviours but the introductory behaviour is shortened. When both snails have acted as male and female in turn (Fig. 4F), no mating attempt occurs for at least 4 hrs. Once total eversion of the preputium is observed, completion of the mating sequence will be the rule, with a few exceptions. Occasionally a female halfway through the copulation sequence climbs some cm above water level and falls down. Then the copulants may become separated. The other exception may occur when the female start ovipositing. Then in some cases the preputium is totally withdrawn and the male moves away. When the deposition of the egg mass is finished, the whole mating sequence may restart, sometimes with the former female in the male role. In other cases the male waits with its preputium partially everted, while attached to the female, until the egg mass is deposited. Then a second, generally successful, attempt follows.

During the entire mating sequence, the partners may have mutual mouth contact from time to time. The longer the periods of isolation, the more frequently this behaviour occurs.

The time relationships between the main male behaviours—turning, partial and total eversion of preputium and intromission—appeared to be variable. In a given experiment, e.g. in some pairs the sequence of mounting, turning and partial eversion of the preputium may take only a few minutes and the total eversion of preputium may occur as much as 90 min later. In other pairs the time between turning and partial eversion of the preputium may take more than an hour whereas it is followed immediately by total eversion of preputium and intromission. In Table I the analysis of the latency from pairing to intromission and the duration of intromission is summarized for four experiments. The coefficient of variation (CV) of the latency of intromission (0.29–0.47) appeared to be much higher than that of its duration (0.08–0.11). A clear trend towards decreasing mean latencies with increasing periods of isolation occurs ($P<0.001$, linear regression). No such trend could be found in the mean duration of intromission, which is fairly constant (36 ± 4 min).

The latency and the duration of intromis-

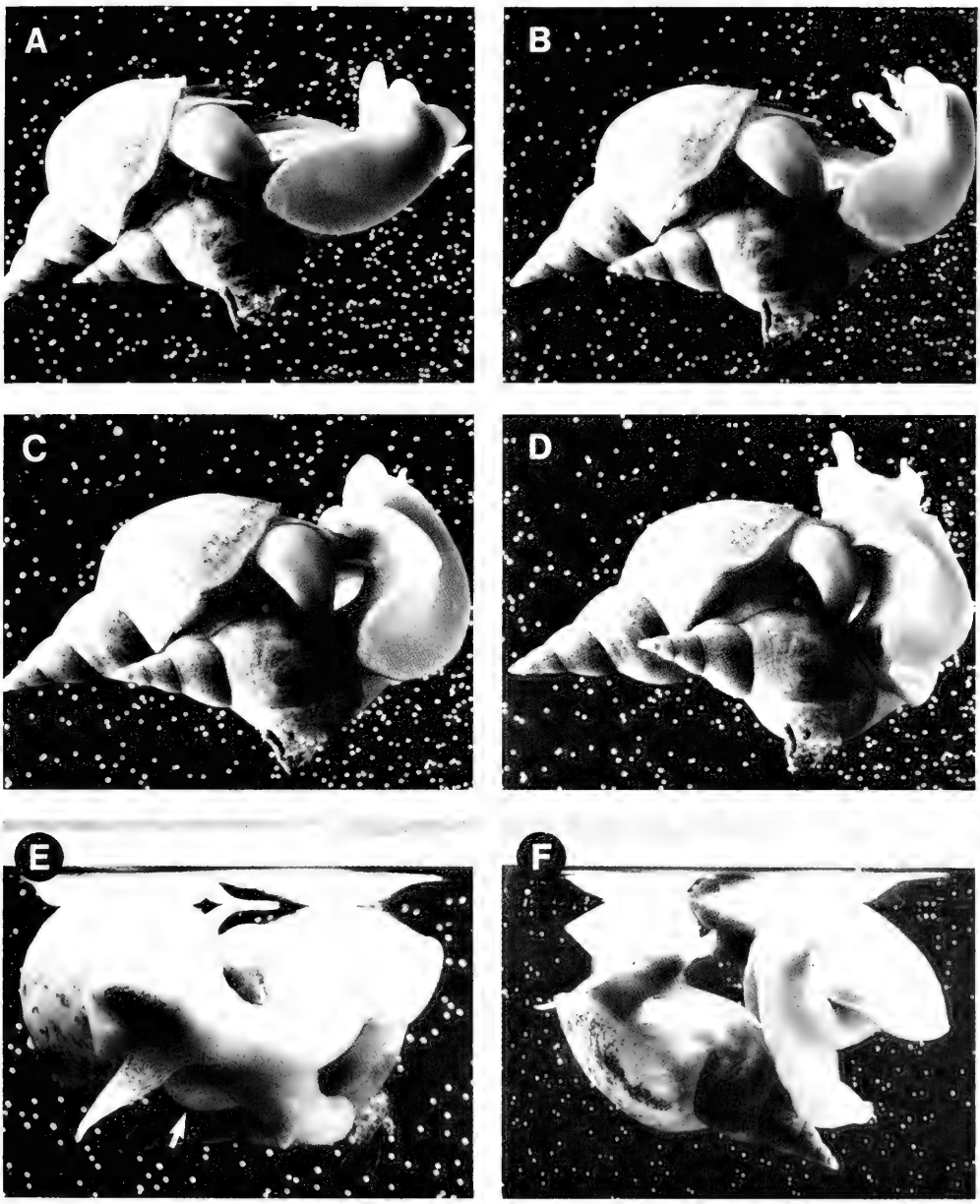

FIG. 4. A. Intromission, male at left. B, C, D. Female (at right) climbing upon the shell of the male while *in copulo*. E. Complex posture of the two snails. From left to right: shell of the male, head/foot of the female, head/foot of the male, shell of the female. Arrow: preputium of the male. F. Separation of the snails after both have acted as male and female in turn.

TABLE 2. Presence of semen in the vagina of female copulants after isolation-induced and spontaneous mating.

	Shell height (mm)	N semen/ N observed	Percentage
Isolation-induced	≥26	59/60[a]	98%
Spontaneous	18-22	28/32	88%
	23-27	23/25	92%
	≥28	23/24	96%

[a]Pooled observations from four experiments.

TABLE 3. Intromission in isolation-induced mating of Operated (partial removal of vas deferens, body wall) and Sham-operated snails. Reciprocal intromission was excluded.

	Intromission					
snails	+	−	Test on homogeneity	G	*df*	P
Op × Op	0	6	Overall	9.889	2	$0.005 < P < 0.01$
Op × SH	3	3	Op × Op/Op × Sh,Sh × Sh	8.604	1	$0.001 < P < 0.005$
Sh × Sh	5	1	Op × Sh × Sh	1.354	1	> 0.4 NS

sion of inexperienced snails (Table 1B) agrees very well with that of experienced snails after 16 days of isolation (Table 1A, fourth row). Intromission occurred in 13 out of 18 pairs in the experienced snails (72%) and in 31 out of 40 pairs in the inexperienced snails (77.5%). These data indicate that copulation ability does not depend on prior experience.

Spontaneous mating

For comparison spontaneous matings in groups of snails were observed. The behaviours described for the isolation-induced matings were also present in spontaneous ones. Reversal of roles after copulation was not observed in these groups, but sometimes a copulating female mounted a third snail and started male behaviour, with the copulating male passively on its shell. Occasionally chains of three snails *in copulo* were encountered, the upper one acting as a male, the middle one acting as its female partner and as a male copulant for the undermost snail. The undermost female sometimes mounted a fourth snail, but chains of more than three animals *in copulo* were not observed.

Sham-copulations were frequently encountered in grouped snails. As in the isolation-induced matings a sham-copulation was generally followed by intromission. This fact was used to determine the duration of intromission in spontaneous copulations. Twelve sham-copulating pairs (shell height 28–33 mm) were followed until the completion of copulation. The mean duration of intromission was 35.8 ± 3.5 min. This value corresponds with that of the isolation-induced copulations (Table 1).

Semen transfer

The data in Table 2 show that in most cases transfer of semen took place. Apparently, larger snails have more success but the relevant differences are not significant ($P > 0.5$, G-test for homogeneity).

Whether the presence of ejaculate in the vagina prevents insemination by a second male, as in some insects (Parker, 1970), was investigated in the following experiment.

Twenty-four snails were paired (12 pairs) after 8 days of isolation. In 10 pairs intromission took place. Immediately after the first mating, the snails were separated to prevent reciprocal copulation. Subsequently 5 pairs consisting of former females were formed and behaviour was observed during the next 150 min. In four pairs intromission took place with durations of 33, 34, 36 and 39 min, respectively. Afterwards the vagina of the ten snails was inspected for semen. In all of them semen was present and in three of the four snails, that had acted twice as a female, the

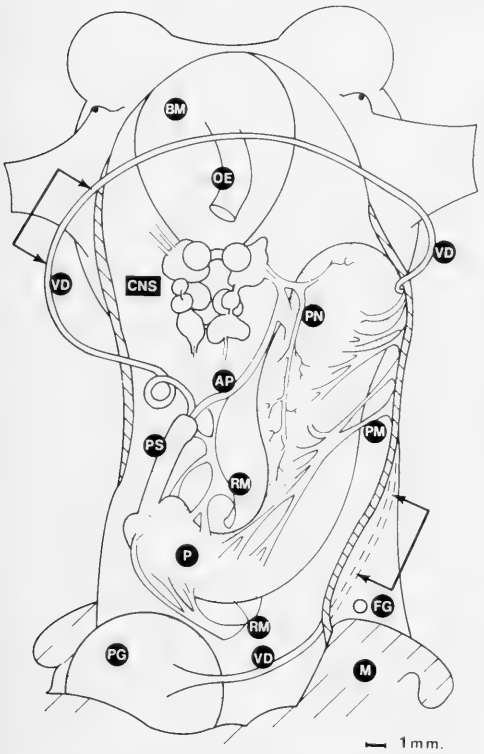

FIG. 5. Dorsal view of the internal organization of the head/foot part of *L. stagnalis.* Arrows refer to the parts of the vas deferens that were surgically removed. AP = artery of the penis, BM = buccal mass, CNS = central nervous system, FG = female gonopore, M = mantle, OE = oesophagus, P = preputium, PG = prostate gland, PM = protractor muscles, PN = penis nerve, PS = penis sheath, RM = retractor muscles, VD = vas deferens.

amount was much larger than ever observed before, indicating that insemination had occurred by the second male. Thus the presence of semen in the vagina does not prevent intromission. Moreover insemination by a second male is highly probable.

Elimination of male mating activity

In pilot studies attempts were made to block semen transfer by surgical removal of different parts of the vas deferens (see Fig. 5 for anatomical details).

Of 16 animals with part of the vas deferens in the head sinus removed, 8 animals survived (50%). Eight days after the operation the animals showed male behaviour (i.e. partial eversion of preputium), but total eversion of preputium and intromission were impaired. Dissection revealed that the loose ends of the vas deferens had grown into the body wall, making total eversion of preputium and intromission impossible.

Ten animals with the part of the vas deferens that runs through the body wall removed, all survived (100%). Animals operated this way not only lacked the possibility of semen transfer, but also failed to show mating activity, even when they were paired after three weeks of isolation. Therefore a more thorough study was made of the effects of this lesion.

The operation was done in 18 snails (Op) and 18 snails were sham-operated (Sh). Afterwards the snails were kept in isolation during a period of 8 days. They were divided in three experimental groups: 6 pairs of operated snails (Op × Op), 6 pairs consisting of an operated and a sham-operated snail (Op × Sh) and 6 pairs of sham-operated snails (Sh × Sh). The behaviour of the pairs was observed during 330 min.

In all pairs mounting was observed. No differences in the latency of mounting between the groups were observed (one-way ANOVA, on log transformed data, $0.10 < P < 0.25$, NS). In the Op × Sh-group all first mountings were made by the sham-operated snail. In the Op × Op-group no copulation behaviour followed, except rarely an incomplete turn. In all pairs of the other groups male behaviour was exhibited, but only by the sham-operated snails. The number of intromissions in the groups with at least one sham-operated snail in the pairs was significantly higher than that in the Op × Op-group (Table 3).

The operated snails did not initiate mating behaviour, but it is conceivable that copulation as a female could induce male behaviour in these snails (cf. *Stagnicola elodes,* Rudolph, 1979a). This hypothesis was rejected by the total absence of any sign of reciprocal behaviour in the operated snails after copulation as a female, whereas the sham-operated snails all exhibited male behaviour after copulation as a female (G-test $P < 0.05$). In all females—operated or sham-operated—semen was present in the vagina.

Removal of part of the vas deferens that runs through the body wall clearly eliminates all male behaviour, but it does not impair the ability to copulate as a female.

DISCUSSION

The mating behaviour of *L. stagnalis* is unilateral: one snail acts as the male and the other as the female. This is the general way of mating in lymnaeid snails (*L. peregra:* Diver *et al.,* 1925; *L. tomentosa:* Boray, 1964; *S. elodes:* Rudolph, 1979a; *L. truncatula:* Smith, 1981).

Characteristic female mating behaviour is absent, whereas male mating behaviour is clear and unmistakable. The basical features are: mounting, turning, eversion of preputium, intromission, withdrawal of preputium and moving away. Several variations are present of which sham-copulation and reciprocation are the most remarkable ones.

In many respects our description of the mating behaviour of *L. stagnalis* is in agreement with the results of Barraud (1957), but there are some contradictions. Firstly, Barraud suggested that, in addition to the copulatory position that we described (position 1, Barraud), intromission is possible from a frontal position (position 2, Barraud). Secondly, at the moment of actual penetration, we did not observe any reaction of the female, whereas he described a contorted retraction of the whole forepart of the female's body at intromission, like we observed in sham-copulations. Thirdly the duration of intromission was 36 $\pm$ 4 min in our experiments, whereas Barraud reported durations of a few min to twelve hours. A fourth difference relates to the success of copulation, which was nearly 100% in our study and low in his. All these differences share a common cause: Barraud did not make a clear distinction between sham-copulation and real copulation. A sham-copulation does resemble a real copulation, but an experienced observer is able to distinguish the two by the characteristic female reaction in sham-copulation. Moreover, occupation of the vagina can in most cases—e.g. with the aid of a mirror—be observed. The high incidence of sham-copulation probably explains all contradictions between our observations and those of Barraud.

Reciprocal copulation behaviour was described extensively for *S. elodes* (Rudolph, 1979a). The readiness to exhibit male behaviour, induced in female copulants, lasts 30–60 min in this snail. When stimulated females are transferred to a third snail during this period, they behave as males. In groups the induced male behaviour of female copulants is probably directed towards a third snail rather than to the partner, since chain copulations but no reversal of roles were observed in groups of snails.

Mouth contact was sometimes encountered during the mating sequence of *L. stagnalis.* It is a common feature of mating behaviour in snails. In helicids (terrestrial pulmonates) courtship commences with mouth to mouth contact (Lind, 1976) and it is a characteristic part of the mating behaviour of the opisthobranch *Aeolidia papillosa* (Longley & Longley, 1984). In *L. stagnalis* it is not an integrated part of mating behaviour.

The mating behaviour of *L. stagnalis* can be broken off in the first stages of the sequence (mounting, turning and occasionally partial eversion of preputium). Once the preputium is totally everted, the sequence will come to completion, although this may take hours.

Mating capability in *L. stagnalis* depends on maturation only, not on prior experience (Table 1). This has been found earlier in *Biomphalaria globosus* (Rudolph, 1983). The duration of intromission was found to be independent of the experience or the period of isolation of the snails. Probably the duration is determined by neuronal timing circuitry, as is assumed to be the case in *A. papillosa* (Longley & Longley, 1984).

After copulation as a female, the readiness to mate as a male as well as a female remains the same. The readiness to mate disappears when both snails have acted as male and female in turn. These observations as well as the decrease in the latency of intromission with increasing period of isolation is in accordance with our model of masculinity and receptivity (Van Duivenboden & Ter Maat, 1985).

Extirpation of the part of the vas deferens that runs through the body wall eliminates all male behaviour in *L. stagnalis.* As yet it is not clear whether this is due to neurological, endocrinological or mechanical blockade. Little is known of mechanisms controlling mating behaviour in other snails. Jeppesen (1976) extirpated various parts of the reproductive system of *Helix pomatia,* but the initiation and the sequence of mating behaviour were not affected. He concluded that mating behaviour is controlled by the central nervous system. The cycle of the mating behaviour in helicids (Lind, 1976, Jeppesen, 1976) seems to depend partly on copulation itself, as in *L. stagnalis* (Van Duivenboden & Ter Maat, 1985) and on mechanical effects of dart-

shooting, a behaviour not present in *L. stagnalis*. The organization of the male and the female reproductive system of *L. stagnalis* (diaulic) is different from that in *Helix* (monaulic) (Visser, 1977, 1981, Geraerts & Joosse, 1984, Tompa, 1984). Therefore a comparison of the extirpations carried out by Jeppesen in *Helix pomatia* with the lesion carried out in this study in *L. stagnalis* is not in order.

In almost all cases, copulation appeared to be successful, i.e. semen could be observed in the vagina of the female. Similar results were found for *S. elodes* (Rudolph, 1979a) and for *B. globosus* (Rudolph, 1979b, 1983). As in *Bulinus,* the presence of semen in the female tract does not prevent intromission and insemination by a second male.

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REPAIRED SHELL DAMAGE IN DEEP-SEA PROSOBRANCH GASTROPODS FROM THE WESTERN NORTH ATLANTIC

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ABSTRACT

We estimated the frequency of repaired shell damage in prosobranch assemblages collected from the continental shelf, upper and lower continental slopes, continental rise and abyssal plain south of New England, U.S.A. Damage was classified as either major (conspicuous breaks generally resulting in displacement of subsequent growth patterns and interruption of sculpture), or minor (fine discontinuities that do not disrupt growth). There is significant variation among regions in the incidence of major and minor damage to prosobranch individuals, but no clear trend with depth. The incidence of species that contain damaged individuals appears to be uniform throughout the 5 regions. Frequencies of major repaired damage in the deep sea (0.08–0.48, median = 0.15 for samples > 200 m in depth) fall within the range of values reported for a variety of shallow-water marine habitats. Known predators of deep-sea snails include fishes, decapods and echinoderms. Their stomach contents indicate very general feeding habits and broad diets. Deep-sea snails and their predators show less evidence of coevolved adaptations than do their shallow-water counterparts.

Key words: deep sea; prosobranchs; predation; repaired shell damage; coevolution.

INTRODUCTION

Biological disturbance by predation has been proposed as a cause of community structure in the deep-sea benthos (e.g. see reviews in Rex, 1981, 1983; Jumars & Gallagher, 1982; Jumars & Eckman, 1983), but its actual importance has been very difficult to determine. One useful approach to studying the effects and geographic patterns of predation in shallow-water faunas has been to measure the incidence of repaired shell damage in gastropods (Vermeij, 1978, 1982a; Vermeij *et al.,* 1982; Bertness & Cunningham, 1981). Gastropods are preyed upon by fishes and decapod crustaceans that are specialized to break open shells to consume the soft parts (Zipser & Vermeij, 1978; Palmer, 1979; Bertness *et al.,* 1981). Unsuccessful predation attempts can result in shell breakage that is repaired by the snail, leaving a distinctive scar.

The record of sublethal shell damage in a population is not correlated in a simple direct way with either the intensity or effectiveness of predation (Schindel *et al.,* 1982). Establishing the exact relationship between predation pressure and the incidence of shell repair requires information on the age structure, reproductive pattern and survivorship of prey, the contribution of predation to overall mortality (Schoener, 1979), the relative abundance of predator and prey, the strength of predators, and the ability of prey to resist or avoid predation (Vermeij, 1982a, 1983). Few such data exist for deep-sea species. Without direct evidence on predator-prey interactions, a low incidence of repair is especially difficult to interpret: predators could be scarce, or, conversely, abundant and extremely efficient at killing prey leaving few scarred individuals, or rarely able to break shells (Schindel *et al.,* 1982; Vermeij, 1982a). In coastal environments, high frequencies of repair generally have been associated with a coevolved predator-prey system in which snails are experiencing potentially lethal predation and have evolved shell architecture to deter it (Schindel *et al.,* 1982; Vermeij, 1982a, 1982b, 1983).

Based upon the scanty evidence available,

[1]Please correspond with M. A. Rex.

TABLE 1. Station data for samples used in the analysis of repaired shell damage in deep-sea gastropods. N is the number of individuals, and S the number of species examined in each station. Frequencies of repaired shells, calculated as the percentage of repaired shells, are given for individuals (%N that show repair) and species (%S, some individual of which shows repair).

							Frequency of repaired shells			
							%individuals		%species	
Region	Station	Latitude	Longitude	Depth (m)	N	S	Major	Minor	Major	Minor
Continental shelf	89	40°1.6′N	70°40.7′W	196	46	5	0.04	0.37	0.40	0.80
Upper slope	88	39°54.1′N	70°37′W	478	158	18	0.08	0.22	0.28	0.72
	105	39°56.6′N	71°03.6′W	530	480	10	0.14	0.26	0.80	0.70
Lower slope	73	39°46.5′N	70°43.3′W	1400	162	14	0.15	0.45	0.43	0.79
	103	39°43.6′N	70°37.4′W	2022	188	17	0.48	0.72	0.53	0.76
	131	39°38.8′N	70°36.8′W	2178	38	5	0.24	0.42	0.80	0.80
Continental rise	76	39°38.3′N	67°57.8′W	2862	63	15	0.21	0.35	0.47	0.60
	126	39°37.3′N	66°45.5′W	3806	66	17	0.20	0.29	0.47	0.53
	77	38°0.7′N	69°16.0′W	3806	109	12	0.13	0.39	0.58	0.92
	85	37°59.2′N	69°26.2′W	3834	239	25	0.13	0.35	0.44	0.64
Abyssal plain	123	37°29.0′N	64°14.0′W	4853	177	9	0.19	0.34	0.67	0.78
	124	37°25.5′N	63°58.8′W	4862	112	7	0.14	0.41	0.29	0.57

Vermeij (1978) cautiously suggested that predation by crushing was unlikely to be important in the deep sea because deep-sea mollusks are small and have poorly developed antipredator armor, and their potential predators apparently lack specialized shell-crushing adaptations. However, in this first analysis of repaired shell damage in deep-sea gastropods, we show that repaired shells are common at bathyal and abyssal depths in the western North Atlantic and occur in most prosobranch species. This suggests that biological and/or physical sources of shell damage are common features of the deep-sea environment.

MATERIALS AND METHODS

The gastropod material

We analyzed the gastropod fraction of 12 epibenthic sled samples (Hessler & Sanders, 1967) collected from the Gay Head-Bermuda Transect south of New England (Sanders *et al.,* 1965). We included only prosobranch species because their early whorls are exposed and can be examined for repaired damage. In most deep-sea opisthobranchs (e.g. *Scaphander, Cylichna, Retusa*) the early whorls are completely obscured by the body whorl, so that sublethal damage in early life cannot be observed. Station data and sample sizes are given in Table 1; maps of sampling localities can be found in Rex (1973, 1976). One sample is from the outer continental shelf, and 11 are from the deep sea (>200 m). Species lists can be found in Rex & Warén (1982). A small number of specimens have been used for other purposes and some shells were too corroded to assess repaired shell damage: we report on 88% of the individuals in the original collections. In total, the material comprised 1838 individuals distributed among 79 species. Only live-collected specimens were used. The complete raw data used in the analysis are provided in the Appendix.

Scoring of repaired shell damage

Identification of predator-induced shell breakage has been necessarily subjective, and has focused on conspicuous damage with clear effects on subsequent growth (see especially Schindel *et al.,* 1982). Vermeij (1982a: 565) distinguished a scar from a normal "'growth line' (interruption of shell growth) by its irregular, usually jagged trajectory"; similar definitions are found throughout the literature on repaired shell damage. Schindel *et al.* (1982) counted repairs in or-

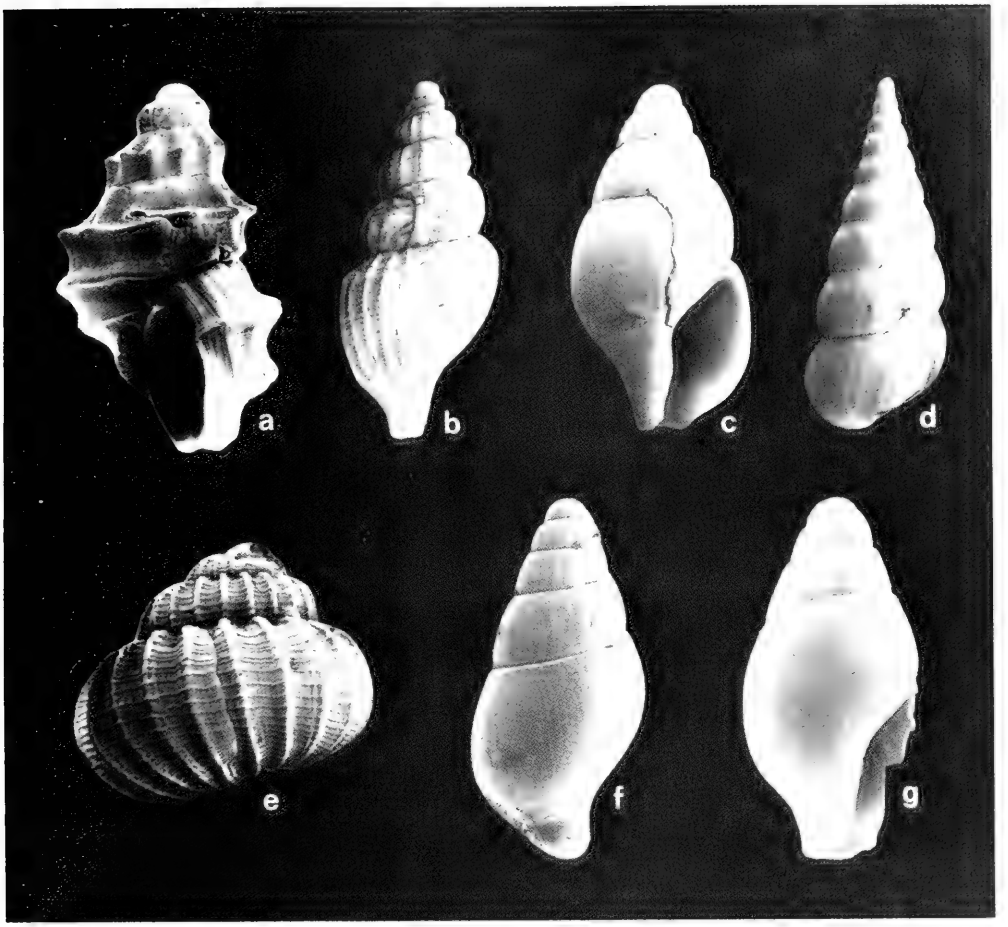

FIG. 1. Examples of major and minor repaired shell damage in prosobranch snails collected from the deep sea of the western North Atlantic. See text for explanation of the injuries. Major damage: a. *Frigidoalvania brychia* (Verrill, 1884), station 105, 3.5 mm; b. *Oenopota bergensis* (Friele, 1886), station 103, 10.3 mm; c. *Mitrella pura* (Verrill, 1882), station 105, 4.0 mm; d. *Aclis walleri* (Jeffreys, 1884), station 103, 3.7 mm. Minor damage: e. *Brookula capensis* Clarke, 1961, station 85, 2.2 mm; f and g. *Mitrella pura* (Verrill, 1882), station 88, 4.0 mm and station 105, 3.5 mm respectively. Sizes represent shell height. Station data are provided in Table 1.

namented species if the sculptural pattern was distorted. Reimchen (1982: 688) categorized breaks in *Littorina* by "extent of injury, from minor disruption of shell growth through major breakages". We compiled data on the frequency of both major and minor breaks.

Some examples of the kinds of repaired damage that we encountered are shown in Fig. 1. We classified as *major* breaks any clear discontinuities in the normal growth pattern of the shell that resulted in temporary post-break displacement of sculptural features (where existing), and large fractures showing evidence of breaking back from the aperture. Fig. 1a shows a displacement of the shoulder ridge by a break at the end of the second whorl. The break is oriented diagonally to the generating curve of the aperture (*sensu* Raup, 1966) and to normal growth lines. Fig. 1b shows a deep break at the end of the third adult whorl that has altered the spacing and shape of post-break axial ribs. Figs. 1c and 1d show jagged irregular breaks that deviate markedly from the shape of normal growth lines, indicating that the lip of the aperture had been broken

back. In most major breaks (e.g. Figs. 1b, c, d) the broken edge is imbricated over the area of resumed growth. Major breaks correspond to the same type of substantial injury that is known to be predator-induced in shallow-water species.

Minor breaks included less extreme damage that caused no obvious distortion of post-break growth. Fig. 1e exhibits a discontinuity of growth, but very little disruption of spacing in either axial or spiral sculpture. Fig. 1f shows a fine irregular fracture without noticeable imbrication. Fig. 1g shows a small break that extends only about one-third of the way across the body whorl. We did not count breakage to the lip of the present aperture (see e.g. Fig. 1g) because this sometimes results from damage incurred during either dredging or sorting and, moreover, is not "repaired".

Minor breaks have been noted, but not included, in analyses by other investigators (e.g. Reimchen, 1982; Vermeij *et al.,* 1980; Vermeij, 1982b; Schindel *et al.,* 1982). They could result from ineffective handling of prey by predators, but could also be due to a variety of physical disturbances, especially in high-energy coastal environments. We felt that the minor breaks were especially interesting in deep-sea species. The deep milieu was long assumed to be very physically and biologically stable, and this view has had important implications for theories of community structure of the deep-sea benthos (Rex, 1981, 1983). However, recent evidence suggests that the deep sea of the western North Atlantic is much more physically and biologically dynamic than once supposed (e.g. Deuser & Ross, 1980; Richardson *et al.,* 1981; Gardner & Sullivan, 1981; Bulfinch *et al.,* 1982; Thistle *et al.,* 1985; Deuser, 1986). Any evidence on variation in the lives of individual organisms bears on the issue of stability in the deep sea.

The Appendix gives the number of shells that showed either major or minor damage for each species.

Although there is a continuum in the severity of repaired damage, we had surprisingly little difficulty scoring breaks as either major or minor. These categories appear to represent two different modal tendencies.

Incidence of repair

The most common measure to quantify the incidence of shell repair has been the average number of scars per shell, calculated as the number of repaired injuries divided by the total number of individuals examined (Vermeij, 1982b). This measure has often been standardized to either size classes of individuals or subsets of whorls, depending on the objectives of the study and limitations of shell form (cf. Currey & Kohn, 1976; Vermeij *et al.,* 1980; Vermeij *et al.,* 1981; Vermeij, 1982a,b; Schindel *et al.,* 1982; Vermeij *et al.,* 1982; Shimek, 1983, 1984). We defined frequency of repair somewhat differently as the percentage of repaired shells, which is computed as the number of individuals having at least one repair divided by the total number of individuals in the sample. This measure has been used by Raffaelli (1978), Elner & Raffaelli (1980), Geller (1983), Bergman *et al.* (1983) and others. It is a more conservative estimate of the frequency of repaired damage than the average number of scars per shell because shells can survive injury more than once (see especially Currey & Kohn, 1976; and Shimek, 1983, 1984). We felt that it was a more appropriate measure for our study because of the tremendous variation in shell form and numerical abundance among the 79 species studied, and because our aim was to get a preliminary overview of the frequency of repaired damage in the deep-sea environment. We computed frequencies for major breaks, minor breaks and combined breaks (either major or minor with redundancies eliminated), for individuals and species.

The twelve stations in Table 1 were grouped for analysis into the five biogeographic assemblages identified by Rex (1977) in a multivariate study of gastropod species composition with depth. These assemblages correspond to five bathymetric regions: the continental shelf (<200 m), upper (200–1000 m) and lower (1000–2000 m) continental slopes, continental rise (2000–4000 m), and abyssal plain (>4000 m). We used a chi-square test with raw data (Siegel, 1956) to test for significant differences between regions in the number of repaired individuals. Fisher-Yates exact probabilities (using the tables of Finney *et al.,* 1963) were used to test for significant differences between regions on the species level, because numbers of species were too low in some regions for valid use of chi-square testing. We also regressed the incidence of breakage in individual samples against depth.

SHELF	2.08	13.70***	2.75	4.14*
U. SLOPE		53.52***	0.45	3.11
L. SLOPE			36.84***	17.31***
RISE				1.06

SHELF	2.57	6.37*	0.01	0.16
U. SLOPE		108.46***	12.52***	12.52***
L. SLOPE			43.68***	28.55***
RISE				0.15

SHELF	0.17	8.52**	0.32	1.22
U. SLOPE		73.43***	10.38**	16.17***
L. SLOPE			27.02***	11.75***
RISE				1.28

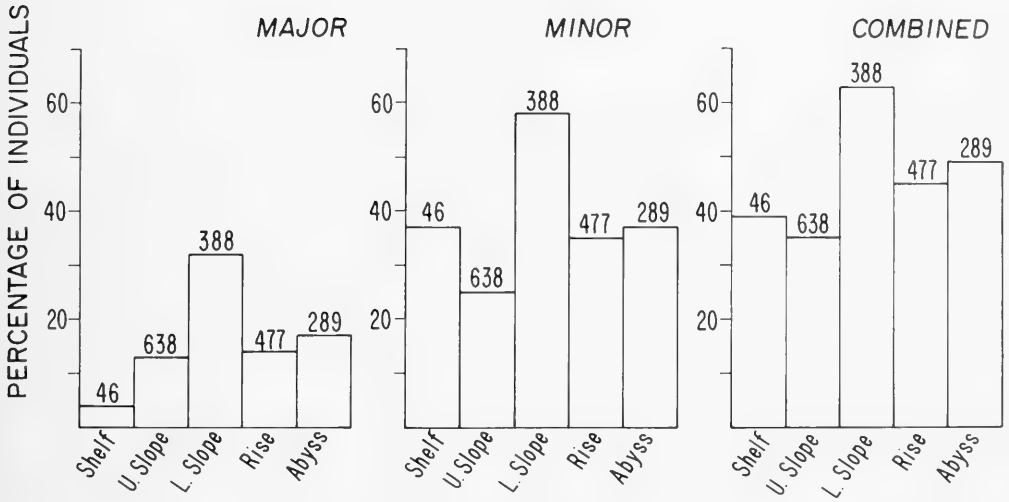

FIG. 2. The percentage of prosobranch individuals that exhibit major, minor and combined (either major or minor with redundancies removed) repaired shell damage in five bathymetric regions of the western North Atlantic. Station data for samples in the regions are given in Table 1. Numbers above the bars represent the number of individuals examined in each region. The tables above the histograms contain the chi-square values (for analyses using raw data) for all possible comparisons of the incidence of damaged individuals among the regions. Columns in the table correspond to the histogram bars directly below them. One, two and three asterisks indicate chi-square values that are significant at the $P < .05$, $P < .01$ and $P < .001$ levels respectively.

RESULTS AND DISCUSSION

Bathymetric patterns in the incidence of repair

Frequency distributions of individuals with major, minor and combined breaks for the five depth regions are shown in Fig. 2. The lower continental slope shows a conspicuously and significantly ($P<.001$) higher incidence (32%) of major breaks among individuals than the other four regions (4–17%). However, this is largely attributable to just one of the lower slope stations (sta. 103), which by itself has a frequency of 48%. Without station 103, the lower slope frequency is 17%, and the only remaining significant difference is between the abyss and the shelf ($P<.05$). When the samples are considered individually, rather than being grouped into regions, there is no significant relationship between the frequency of major breaks and depth of the samples by using either linear regression ($Y = 15.198106 + 0.000993X$, $r = 0.1518$, d.f. = 10, $P>.10$) or parabolic regression ($Y = 4.057192 + 0.015960X - 0.000003X^2$, $r = 0.5983$, d.f. = 9, $P>.05$).

Minor breaks among individuals are roughly twice as common as major breaks from the upper slope to the abyss and about ten times more common on the shelf (Fig. 2). The lower slope shows a higher frequency of minor breaks (58%) than the other four regions (25–37%). Again this is partly due to station 103 which has a frequency of 72%; although when station 103 is removed from the analysis, significant differences persist between the lower and upper slope ($P<.001$)

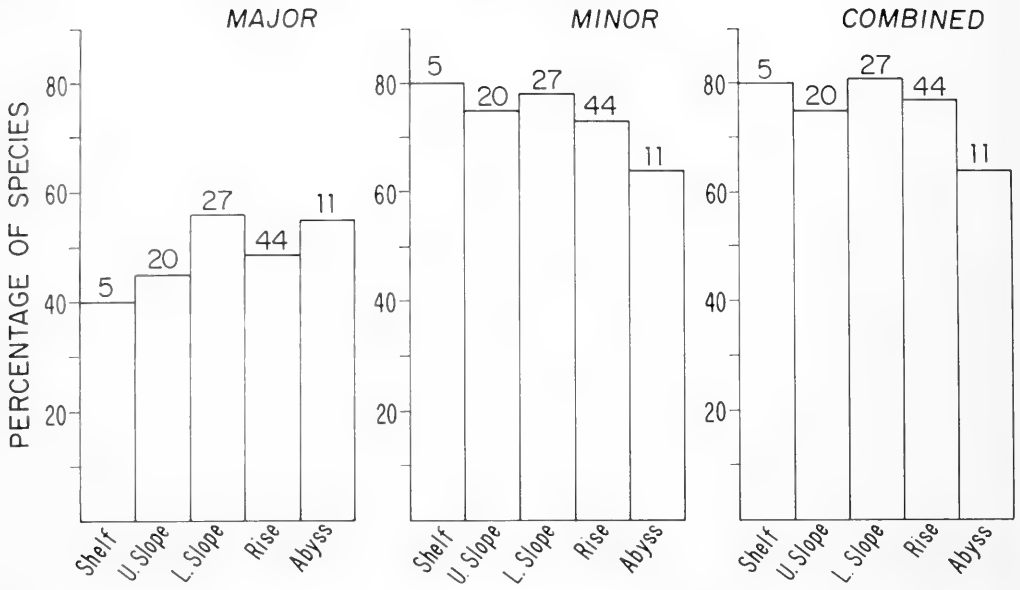

FIG. 3. The percentage of prosobranch species that exhibit major, minor and combined (either major or minor with redundancies removed) repaired shell damage in five bathymetric regions of the western North Atlantic. Station data for samples in the regions are given in Table 1. Numbers above the bars represent the number of species examined in each region. The incidence of species showing damage was compared among the regions by using Fisher-Yates exact probabilities (tables of Finney *et al.*, 1963). There were no significant differences among regions for any of the three categories of repaired damage.

and between the lower slope and continental rise ($P<.05$). The upper slope has a significantly lower frequency than either the rise or the abyss ($P<.001$). Neither linear nor parabolic regressions reveal any pattern in the frequency of minor breaks with depth when individual samples are analyzed ($Y = 36.837998 + 0.000485X$, $r = 0.0648$, d.f. = 10, $P>.10$; and, $Y = 27.330179 + 0.013258X - 0.000003X^2$, $r = 0.4368$, d.f. = 9, $P>.10$ respectively). Clearly, the combined distribution is influenced most by the incidence of minor breaks.

The percentage of species showing repaired shell damage is shown in Fig. 3. There are no significant differences among any of the regions for either major, minor or combined breaks. Similarly, linear and parabolic regression models show no pattern in the frequency of either major or minor breaks with depth when individual samples are analyzed (highest $r = 0.2935$, $P>.10$). Most species in all of the regions contain individuals with some degree of repaired damage, which is especially remarkable since 54% of the species in the collections studied are represented by five or fewer individuals, and 26% of the species are represented by only a single individual.

In addition to the previously-mentioned problems in assessing and measuring repaired damage, there are other difficulties with interpreting its incidence along depth gradients. One is that physiological activity rates may decline exponentially with depth. For example, Smith (1978) showed that benthic community respiration drops three orders of magnitude from the continental shelf to the abyss in the western North Atlantic. If growth rates are correspondingly slower and longevity higher at greater depths (e.g. Turekian *et al.*, 1975), then snails from deeper regions have longer to experience shell damage. The effect of this, using our method, would be to overestimate progressively the actual frequency of repair with increasing depth. It is unknown whether rates of inflicting damage and growth rates of snails vary in some proportional way with depth. Another complication is that faunal density and biomass vary with depth and differ among taxa and ecological assemblages (Rex, 1983). It is impossible to say with any precision how variation in community structure is

related to intensity of predation on snails by various potential predators. Since there is no direct observational evidence on predator-prey interactions, we cannot address critically the behavioral implications of "unsuccessful predation" (see Vermeij, 1982c, 1985; Sih, 1985). The complexity and uncertainty of the situation limit us to fairly general conclusions.

Interregional comparison of repaired damage

How do frequencies of shell repair found in deep-sea gastropods compare with frequencies observed in shallow-water environments? There are many difficulties with making such comparisons in a critical way, including differences in sample sizes, numbers of species studied, habitat type, shell architecture among the species and methods used to assess the frequency of repair. Only major breaks can be considered, since minor breaks have been excluded from other studies. Regional variation in frequency of repair can be estimated by comparing medians and ranges of frequencies among sampling sites. Table 2 provides these data for living temperate and tropical coastal faunas.

The deep-sea (>200 m) samples have a median frequency of 0.15 and a range of 0.08–0.48 (Tables 1 and 2). If the outer shelf sample (sta. 89) is included, the median is 0.145. Much of what is known about shell repair in the temperate North Atlantic comes from extensive studies on species of *Littorina,* a rocky intertidal group that is subject to predation by durophagous crabs (especially *Carcinus maenas*), and to crushing by boulders. Medians for repair frequency in littorinids are ≤0.09. Median frequencies in Pacific temperate prosobranchs appear to be higher, but these populations have not been sampled as extensively and the medians are well within the ranges of values reported for Atlantic littorinids. Temperate terebrids show a median of 0.31. Terebrids have many-whorled, tall-spired shells. Populations can have frequencies of repair that are an order of magnitude higher than less-turreted species. Terebrid shell shape is apparently an adaptation to thwart predation; snails can withdraw up into the shell beyond the crabs' ability to peel back from the aperture (Vermeij *et al.,* 1980). The median for all of these temperate populations, including the terebrids, is 0.09 (range 0–0.96). Although data from Vermeij's (1982a) extensive analysis of *Littorina littorea* make up most of the sample (and therefore might be expected to strongly affect the calculated median), when this study is omitted the median remains very similar at 0.10. The median without *L. littorea* and the terebrids is 0.08 (range = 0–0.50).

Frequencies in tropical populations tend to be higher. This can be seen for the thaidids (Vermeij, 1978), for which data were not presented to enable us to calculate medians, but which show a clear shift in range to higher values at tropical sites. The tropical study most comparable to the deep-sea data presented here and the temperate data discussed above is Vermeij's (1982b) analysis of snail faunas from 14 localities in the Pacific. The median frequency is 0.28. The overall pattern to emerge is an increase in median frequency of repair from temperate coastal environments (0.10) to the deep sea (0.15) to tropical environments (0.28). A more conservative conclusion is that the deep-sea gastropod fauna shows frequencies of repaired shell damage that fall within the range of those found in coastal faunas.

Predation on deep-sea gastropods

Gastropods have been found in the stomach contents of many deep-sea fishes including clupeoids (Mauchline & Gordon, 1983), chimaeriformids, halosaurids, gadids, zoarchids (Sedberry & Musick, 1978) and macrourids (Haedrich & Polloni, 1976; McLellan, 1977; Mauchline & Gordon, 1984). Deep-sea demersal and benthopelagic fishes that rely on benthic prey tend to be highly euryphagous (McLellan, 1977; Sedberry & Musick, 1978). Gastropods are not common prey items, usually making up about one percent or less of prey individuals when they are found at all in fish stomach contents. Bright (1970), in his study of the stomach contents of 36 species of deep-sea fishes, expressed surprise that gastropods comprised such a small proportion (one percent) of prey items. The explanation is that deep-sea snails live at low density. In a quantitative sampling study of the deep-sea benthos south of New England (>200 m), snails were encountered in 56% of the samples taken, and made up only 0.1–2.0% (median 0.4%) of the macrobenthos in samples where they occurred (Sanders *et al.,* 1965). Their incidence in stomach contents of deep-sea fishes corresponds roughly to their availability. The presence of shell fragments in stomach con-

TABLE 2. Comparison of frequencies of repaired shell damage among deep-sea, temperate coastal and tropical coastal gastropod faunas. PRS and ANS indicate percentage of repaired shells and average number of scars respectively; see text for explanation. The median frequency of repair is calculated among collecting sites for each study.

Reference	Region	Habitat	Fauna	# of sites	Frequency of repair		
					Method	Median	Range
This study	Deep-sea Western North Atlantic	Soft bottom	79 Species	11	PRS	0.15	0.08–0.48
Geller (1983)	Temperate Eastern North Pacific	Rocky Intertidal	*Tegula funebralis*	3	PRS	0.29	0.04–0.50
			Nucella emarginata	4	PRS	0.10	0.06–0.20
Bergman *et al.* (1983)	Temperate Eastern North Pacific	Seagrass Beds	*Alia carinata*	2	PRS	0.25	0.15–0.36
Raffaelli (1978)	Temperate Eastern North Atlantic	Rocky intertidal	*Littorina rudis*	24	PRS	0.06	0–0.48
Elner & Raffaelli (1980)	Temperate Eastern North Atlantic	Rocky intertidal	*Littorina rudis*	3	PRS	0.06	0.03–0.32
			Littorina nigrolineata	3	PRS	0.08	0.07–0.11
Reimchen (1982)	Temperate Eastern North Atlantic	Rocky intertidal	*Littorina mariae*	5	PRS	0.09	0.05–0.46
			Littorina obtusata	5	PRS	0.02	0.01–0.44
Vermeij (1982a)	Temperate North Atlantic	Rocky intertidal	*Littorina littorea*	186	ANS	0.08	0–0.59
Vermeij (1982b)	Tropical Pacific	Soft bottom	53 species	14	ANS	0.28	0–0.82
Vermeij *et al.* (1980)	Tropical Indo-Pacific and Atlantic	Soft bottom	Terebridae (61 spp.)	144	ANS	0.72	0–9.19
	Temperate Pacific and Atlantic	Soft bottom	Terebridae (14 spp.)	20	ANS	0.31	0–0.96
Vermeij (1978)	Tropical Eastern Pacific	Rocky intertidal	Thaidid snails (4 spp.)	17	ANS	—	0.09–0.63
	Temperate Eastern Pacific	Rocky intertidal	Thaidid snails (8 spp.)	21	ANS	—	0–0.29

tents (Sedberry & Musick, 1978) indicates that fish predation can cause shell damage.

Decapods are poorly represented in the deep sea, compared to coastal waters (Hessler & Wilson, 1983), but Lagardère (1977a,b) has shown that mollusks, including snails, are common (0–41%, median = 11%) prey items in the stomach contents of many deep-sea decapods. Snails are eaten by deep-sea lobsters, and members of three families of deep-sea shrimps (Penaeidae, Pandalidae and Crangonidae). There are brachyuran crabs living on the continental slope south of New England which could crush snail shells in the same way that *Carcinus maenas* crushes *Littorina* (e.g. Vermeij, 1982a). However, except for the red crab *Geryon quinquidens,* whose bathymetric range extends to 1670 m, most species occur at depths less than 600 m (Wenner & Boesch,

1979). Lagardère (1977a,b) found bivalves in the stomachs of deep-sea *Geryon* and *Pagurus,* suggesting that other hard-shelled prey could be consumed. As with deep-sea fishes, the diets of deep-sea decapods appear to be very generalized (Lagardère, 1977a,b).

Deep-sea echinoderms also prey on snails. Most deep-dwelling ophiuroids are unselective omnivores (Pearson & Gage, 1984). Their stomach contents frequently include whole snails (0–2% of diet items) and shell fragments (Litvinova & Sokolova, 1971; Pearson & Gage, 1984). Carey (1972) found snails among stomach contents in 6 out of 26 species of deep-sea asteroids; snails were a dominant food source for 4 species. He pointed out that, in contrast to shallow-water asteroids, deep-sea species tend to have highly generalized feeding habits. The incidence of omnivorous species increases from 0% in the sublittoral zone to 71% in the abyssal zone.

Vermeij (1978) was correct in saying that deep-sea predators are not highly adapted to crush hard-shell prey. There appear to be no potential predators in deep water comparable to tropical brachyuran crabs like *Calappa* that peel open snail shells with their massive specialized chelae (Shoup, 1968), or to the spiny puffer fish *Diodon* that uses reinforced jaws to crush snails (Palmer, 1979). However, deep-sea snails are consumed by a wide variety of more generalized fishes, decapods and echinoderms which are capable of causing shell damage, and major breaks in deep-sea snails resemble those caused by crushing from fishes and crabs in coastal environments.

Whether what we have termed minor breaks result from a special and different set of causes is purely conjectural. Since most predators that are known to consume snails appear to be unspecialized megabenthic croppers (*sensu* Dayton & Hessler, 1972), it is easy to imagine both major and minor breaks resulting from ineffective prey handling. Biological activities like the "mud-grubbing" foraging behavior of macrourid fishes (McLellan, 1977) and burrowing of red crabs (Hecker, 1982) could also inflict damage, although these are probably most prevalent from upper to mid-bathyal depths. A possible source of minor damage at lower bathyal and abyssal depths in the western North Atlantic is strong near-bottom currents which resuspend and transport sediments (Richardson *et al.,* 1981; Bulfinch *et al.,* 1982) and could probably tumble snails, chipping the outer lips of their apertures. Turbidity currents and sediment slumps (Bulfinch *et al.,* 1982) may have a similar effect at bathyal depths. It is also conceivable that annual and long-term variation in trophic input from the surface (Deuser, 1986) and resuspension and transport of sediments in benthic storms (Gardner & Sullivan, 1981) sometimes result in nutrient depletion of sediments that is especially severe even by deep-sea standards. Weakened calcification of the outer lips of shell apertures during such periods might make them more subject to damage, or result in distinct growth checks that are more pronounced than normal growth lines on the shell.

Implications for coevolution

Vermeij (1978, 1983) has reviewed aspects of shell form that serve as antipredator adaptations. Thick shells and bold sculpture of forms like *Frigidoalvania brychia* (Fig. 1a) have been shown experimentally to be effective antipredator devices (Palmer, 1979). However, this type of shell armor is uncommon in deep-sea snails. In general, they are not heavily calcified and have more delicate sculpture. Many archaeogastropods are umbilicate, which would make them more vulnerable to predation (Vermeij, 1978). Deep-sea snails are also quite small, generally less than one centimeter and frequently only a few millimeters in length.

It is likely that energy limitations in the deep sea have scaled down the physical strength of predators and the resistance of hard-shelled prey. Another limitation to both mollusks and some of their invertebrate predators is that calcium carbonate becomes more soluble with decreased temperature and increased pressure. Dissolution of calcium carbonate selects for more efficient use of calcite and aragonite in shells which is often manifested by thinner shells and constraints on shell form (Graus, 1974). Our future research plans include inter- and intraspecific analyses of Vermeij's antipredator morphologies and application of Graus' calcification index to determine the relative importance of predation and calcium carbonate availability for shell form in deep-sea snails.

The highly coevolved predator-prey systems found in shallow water, where reciprocal selection has led to very powerful and specialized shell-crushing structures in predators

and resistant antipredator architecture in snail shells, appear not to have developed as extensively in the deep sea. The predators of deep-sea snails appear to be unspecialized consumers with very general diets. Similarly, snails, for the most part, have not evolved elaborate and specific defense armor. They are subjected to unsuccessful attacks from potentially lethal predators. Predation and, or, physical disturbance strong enough to break shells are common features of the deep-sea environment.

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APPENDIX

Locality and species	Total number examined	Number with major repaired damage	Number with minor repaired damage
Station 89, 196 m			
Onoba pelagica	37	1	13
Mitrella pura	5	1	1
Eulimella unifasciata	2	0	2
Aclis tenuis	1	0	1
Aclididae sp.	1	0	0
Station 88, 478 m			
Mitrella pura	31	4	7
Anachis haliaeeti	30	4	8
Solariella obscura	19	0	3
Frigidoalvania brychia	17	2	3
Pusillina harpa	11	0	1
Oenopota ovalis	8	0	1
Lepetella tubicola	10	0	0
Pusillina pseudoareolata	6	0	2
Admete contabulata	5	2	1
Lissospira sp. A	5	0	3
Cocculinidae sp. A	5	0	0
Colus pygmaeus	3	0	2
Onoba pelagica	3	0	0
Turridae sp. A	1	0	1
Cerithiella whiteavesii	1	0	1
Aporrhais occidentalis	1	0	0
Aclis tenuis	1	1	1
Calliotropis sp. A	1	0	0
Station 105, 530 m			
Frigidoalvania brychia	155	33	50
Mitrella pura	163	22	36
Pusillina harpa	89	3	16
Onoba pelagica	44	5	15
Solariella obscura	21	1	6
Aclis walleri	3	2	2
Admete contabulata	2	0	0
Colus pygmaeus	1	0	1
Anachis haliaeeti	1	1	0
Taranis morchi	1	1	0
Station 73, 1400 m			
Oenopota ovalis	64	0	23
Aclis walleri	30	19	29
Buccinidae sp. A	23	1	4
Cyclostrematidae sp. A	14	0	2
Gymnobela sp. A	9	0	7
Natica sp. A	4	0	0
Oenopota graphica	4	0	2
Pleurotomella packardi	4	1	1
Cerithiella whiteavesii	2	0	2
Admete contabulata	2	1	1
Lissospira sp. A	1	0	0
Benthonella sp. A	1	1	1
Gymnobela sp. D	1	1	1
Bathysciadium costellatum	3	0	0
Station 103, 2022 m			
Aclis walleri	101	69	94
Oenopota ovalis	20	7	6
Oenopota graphica	16	3	13
Boreotrophon abyssorum	10	3	7

Appendix Continued

Locality and species	Total number examined	Number with major repaired damage	Number with minor repaired damage
Station 103, 2022 m (cont.)			
Theta chariessa	8	3	5
Buccinidae sp. A	11	1	1
Pleurotomella packardi	5	0	1
Cerithiella whiteavesii	4	1	3
Lora harpularia	3	2	1
Pleurotomella sandersoni	1	0	1
Rissoidae sp. A	1	0	1
Cancellariidae sp. A	2	0	1
Gymnobela brevis	1	0	0
Gymnobela sp. C	1	0	1
Aclididae sp. A	1	0	0
Gymnobela sp. A	1	1	0
Pleurotomella sp. A	2	0	0
Station 131, 2178 m			
Lissospira sp. A	18	2	4
Rissoidae sp. A	9	3	6
Aclis walleri	4	3	4
Cyclostrema smithi	6	0	2
Boreotrophon abyssorum	1	1	0
Station 76, 2862 m			
Benthomangelia antonia	37	7	11
Gymnobela frielei	5	1	2
Boreotrophon abyssorum	5	0	0
Pleurotomella packardi	3	0	3
Gymnobela sp. E	1	0	1
Gymnobela sp. C	2	0	0
Tacita sp. A	2	1	0
Pleurotomella sandersoni	1	0	0
Gymnobela tincta	1	0	1
Theta chariessa	1	1	1
Pleurotomella sp. A	1	1	1
Gymnobela bairdii	1	1	0
Gymnobela sp. B	1	0	1
Gymnobela sp. F	1	1	1
Benthomangelia sp. A	1	0	0
Station 126, 3806 m			
Benthomangelia antonia	18	2	7
Solariella sp. A	10	0	1
Benthonella tenella	8	1	1
Tacita sp. A	8	5	4
Pleurotomella sandersoni	5	0	1
Lissospira sp. C	3	1	0
Gymnobela tincta	3	1	2
Theta lyronuclea	1	1	1
Omalogyra sp. A	1	0	0
Epitonium nitidum	2	0	0
Cocculinidae sp. B	1	0	0
Leucosyrinx sp. A	1	1	1
Gymnobela sp. F	1	1	0
Gymnobela sp. G	1	0	0
Buccinidae sp. A	1	0	1
Omalogyra sp. B	1	0	0
Benthomangelia sp. A	1	0	0
Station 77, 3806 m			
Benthomangelia antonia	39	1	12
Benthonella tenella	23	4	13

Appendix Continued

Locality and species	Total number examined	Number with major repaired damage	Number with minor repaired damage
Station 77, 3806 m (cont.)			
Boreotrophon abyssorum	11	1	5
Pleurotomella sandersoni	12	5	2
Benthobia tryoni	8	1	3
Pleurotomella lottae	5	1	1
Brookula sp. A	3	0	0
Gymnobela sp. F	3	0	2
Typhlomangelia sp. A	1	0	1
Leucosyrinx sp. B	1	1	1
Tacita sp. A	1	0	1
Gymnobela sp. E	2	0	1
Station 85, 3834 m			
Benthonella tenella	65	9	25
Benthomangelia antonia	44	2	12
Lissospira sp. D	24	1	6
Adeorbis umbilicatus	20	2	5
Pleurotomella sandersoni	15	4	5
Pleurotomella lottae	13	2	9
Benthobia tryoni	12	1	2
Brookula sp. A	7	2	4
Pleurotomella sp. B	2	0	2
Theta lyronuclea	2	1	2
Drilliola sp. A	4	2	4
Gymnobela sp. B	2	0	0
Benthomangelia sp. A	5	0	3
Turridae, sp. B	2	0	2
Boreotrophon abyssorum	2	0	0
Gymnobela sp. F	2	0	0
Epitonium sp. B	2	2	0
Tacita sp. A	2	0	1
Xanthodaphne sigmoidea	1	0	0
Belomitra sp. A	1	0	1
Turridae sp. C	1	0	0
Tharsiella sp. A	1	0	0
Lissospira sp. B	5	0	1
Cocculinidae sp. B	4	0	0
Gymnobela curta	1	0	0
Station 123, 4853 m			
Benthonella tenella	116	24	50
Adeorbis umbilicatus	21	1	2
Lissospira sp. D	20	3	4
Pleurotomella sp. B	6	1	0
Gymnobela sp. F	6	4	1
Theta lyronuclea	4	0	1
Drilliola sp. A	1	0	1
Cocculinidae sp. B	2	0	0
Belomitra sp. A	1	1	1
Station 124, 4862 m			
Benthonella tenella	82	15	41
Adeorbis umbilicatus	23	1	2
Drilliola sp. A	2	0	2
Lissospira sp. D	2	0	0
Gymnobela sp. F	1	0	1
Benthobia tryoni	1	0	0
Xanthodaphne sp. B	1	0	0

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SPERMATOGENESIS IN *ONCOMELANIA HUPENSIS QUADRASI*, A MOLLUSCAN HOST OF *SCHISTOSOMA JAPONICUM*

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ABSTRACT

Hepatotestes of laboratory reared male *Oncomelania hupensis quadrasi* (25 wk old) were processed for transmission electron microscopy, and spermatogenesis was studied. Within testicular acini are spermatogonia, sperm, numerous spermatocytes and spermatids in various stages of differentiation. Mature sperm are filiform and contain a homogeneous mass of nucleoprotein, spiralled around the head shaft. The Nebenkern consists of seven giant mitochondria which share a common, outer mitochondrial membrane, with their inner membranes remaining intact. Head shaft and flagellar axoneme show the typical cartwheel pattern of 9 + 2 microtubules. A single Golgi-complex was noted consistently in developing cells, whose granular secretions contribute to acrosome formation. Proximal and distal centrioles, seen only in early stages of spermatid differentiation, apparently contribute to the formation of the intranuclear and flagellar axoneme. A row of microtubules (= manchette) surrounds the non-helicoidal, homogeneous mass of nucleoprotein and developing Nebenkern of elongate spermatids. While microtubules around the nucleus are located away from the nuclear membrane, in the Nebenkern, these microtubules closely appose the outer mitochondrial membrane. Microtubules are either absent or widely scattered in mature sperm. Whether these microtubules participate in determining the final corkscrew form of the sperm is not clear. While a few biflagellate sperm were noted, atypical forms reported in other prosobranch snails such as apyrene and oligopyrene sperm, were not observed. Sertoli cells with many electron dense bodies and prominent nuclei are confined to the acinar wall.

Key words: spermatogenesis, prosobranchia, *Oncomelania hupensis quadrasi*.

INTRODUCTION

Studies on the process of spermatogenesis at the ultrastructural level have been reported in various prosobranch snails such as *Viviparus* spp. (Hanson *et al.,* 1952; Gall, 1961), *Cipangopaludina* spp. (Yasuzumi & Tanaka, 1958; Yamasaki, 1966), *Epitonium tinctum* (Bulnheim, 1968), *Nucella lapillus* (Walker, 1970), *Littorina sitkana* (Buckland-Nicks & Chia, 1976), *Ocenebra erinacea* (Féral, 1977), *Colus stimpsoni* (West, 1978), *Bithynia tentaculata* (Kohnert, 1980), and *Lambis lambis* and *Conomurex luhuanus* (Koike & Nishiwaki, 1980). While working on *Oncomelania hupensis quadrasi* infected with *Schistosoma japonicum,* we observed total or partial loss of testicular tissue. Although destruction of the gonads has been reported before in some mollusks infected with trematode parasites (Rees, 1934, 1936; Pratt & Barton, 1941; Sullivan *et al.,* 1985), there are no published reports of such damage in oncomelanian snails. To date, the process of spermatogenesis has not been critically described in any strains of *Oncomelania hupensis.* We anticipate that the present description in mature, uninfected *O. h. quadrasi* may eventually be used for comparison of spermatogenesis in infected male snails which exhibit parasitic castration. Furthermore, these data will contribute to our general knowledge of sperm formation in prosobranch snails.

MATERIALS AND METHODS

Snail cultivation

Oncomelania hupensis quadrasi snails were reared in the laboratory following the cultivation techniques of Van der Schalie & Davis (1968) and French (1974) with some modifications. Plastic lined aluminum trays (17 × 24″), half filled with sterile muddy soil and water, were exposed to two-40W cool white fluorescent lights at least 6 hr/day, to stimulate growth of blue-green algae as snail

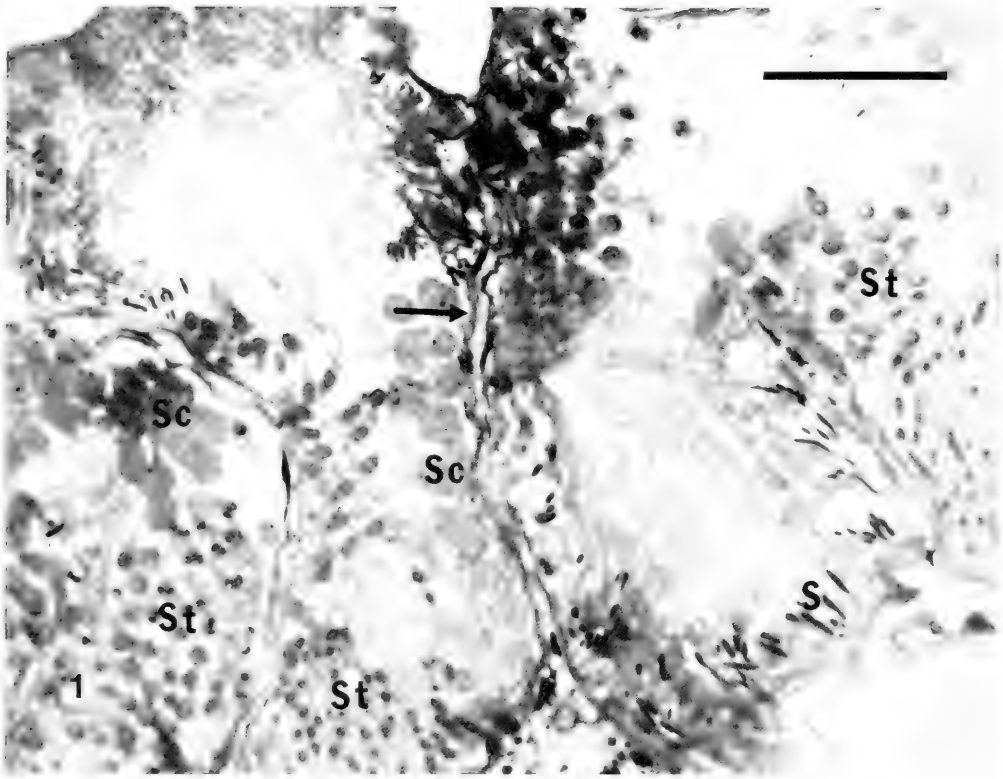


FIG. 1. Section of testis showing testicular acini with spermatocytes (Sc), various stages of spermatids (St) and sperm (S). Acinar wall (arrow). Bar = 40 μm

food. Filaments of *Nostoc* sp. were added to the culture trays to supplement snail diet. Aerated tap water was added when necessary.

Electron microscopy

Six mature male *O. h. quadrasi* (25 wk old) were cleaned of soil particles with a fine brush, lightly crushed and their shells carefully removed under a stereoscope. Hepatotestes were cut from the rest of the snail body, fixed in 3% glutaraldehyde in 0.15 M sodium cacodylate buffer (pH 7.4) overnight at 4°C. Tissues were washed in 0.2 M cacodylate buffer four times at 30 min intervals, post-fixed in 1% buffered osmium tetroxide for 1 hr at 10°C, and then stained with 2% uranyl acetate in 10% ETOH for 45 min. Specimens were dehydrated in serially graded ethanol (30, 50, 70, 80, 95%) for 10 min each wash, followed by 100% ethanol and propylene exode (2 changes each) for 10 min. Tissue infiltration employing 50:50 parts propylene oxide and Spurr resin for 6 hr was followed by embedding in 100% Spurr resin in plastic capsules, polymerized at 60°C for 48 hr.

Sections 8–10 nm thick were cut with a diamond knife using ultramicrotomes (Reichert OM U3 and Sorvall MT 2-B), then stained with lead acetate for 3 min. Sections were observed using a 9S-2 Zeiss and a Phillips 300 electron microscopes.

RESULTS

Testes of normal, mature male *O. h. quadrasi* show all the developmental stages of spermatogenesis, including spermatogonia, spermatocytes, spermatids and sperm within testicular acini (Fig. 1). The acinar wall has an inner layer of Sertoli cells, which is delimited from the outer germinal epithelium by a narrow, less electron dense layer. Sertoli

cells have prominent nuclei and highly granular cytoplasm containing numerous electron dense bodies of varied shapes and sizes. The germinal epithelium has a layer of flattened cells, with large nuclei and scanty cytoplasm (Figs. 25, 26).

Very few spermatogonia were observed near the acinar wall. They are relatively smaller than spermatocytes, measuring 4.5–6.0 μm diam, (nuclear diam from 3.5–5.4 μm). Their scanty cytoplasm contains few cytoplasmic organelles such as endoplasmic reticulum, mitochondria and Golgi material. A single nucleolus was noted in some cells (Fig. 2).

Spermatocytes are spherical to irregular in shape, with nuclei containing patchy chromatin with or without nucleoli (Fig. 3). Primary spermatocytes are generally larger than spermatogonia, measuring 6.2–8.7 μm diam with nuclei ranging from 4.0–5.8 μm across. They contain increased numbers of mitochondria with early signs of clustering; both the Golgi material and endoplasmic reticulum are conspicuous as well (Fig. 3).

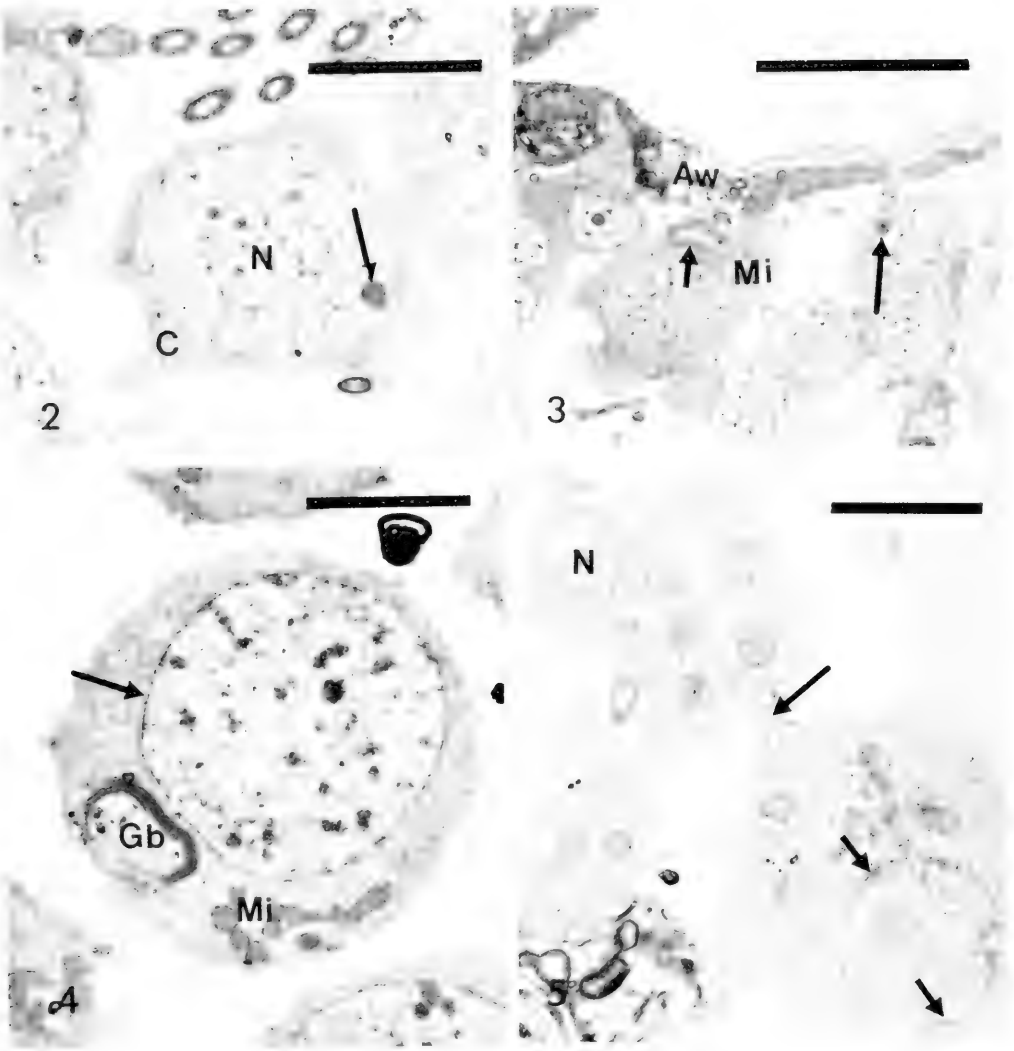
Primary and secondary spermatocytes are often difficult to differentiate. However, secondary spermatocytes can be recognized by the presence of well-formed Golgi body and larger mitochondria developing from the fusion of smaller ones, forming a cluster at one end of the cell (Figs. 4, 5). Cytoplasmic bridges were seen between some late secondary spermatocytes, indicating delayed cytokinesis, and nuclei of some cells have basal and apical thickenings (Fig. 5).

Spermiogenesis is divided arbitrarily into four stages (Buckland-Nicks & Chia, 1976; Eckelbarger & Eyster, 1981), based on general nuclear shape and chromatin condensation, development of giant mitochondria into a Nebenkern, formation of Golgi-complex and acrosome and the axonemal complex.

Stage A (pre-cup stage) spermatids are irregularly shaped, measuring 2.4–7.3 μm diam, with subspherical nuclei, 2.4–3.1 μm diam in either central or eccentric position (Fig. 6). The antero-posterior axis of the cell is established early, with the formation of a basal and apical thickening of electron opaque material at opposite ends of the nucleus (Figs. 6, 7). The basal plate invaginates, forming a small cavity or indentation (Figs. 7, 8). Meanwhile, the nuclear material begins to condense into granular chromatin and aggregates to form lateral patches on the inner nuclear envelope, leaving a somewhat less electron dense space in the basal or apical area (Figs. 6, 7). The cytoplasm has numerous cisternae of endoplasmic reticulum, large mitochondria, and a well-developed Golgi body with stacked saccular membrane and granular secretions (Figs. 8, 9). During the pre-cup stage, Golgi-complex and mitochondria are not necessarily positioned according to where they occur in later developmental stages along the antero-posterior axis of the cell.

The change in cell size from stage A to stage B (cup-shaped) is slight, and many spermatids have features common to both stages. The small shallow indentation in the center of the basal end of the nucleus, normally observed during the pre-cup stage, grows deeper with the insertion of a cap-like terminal end of the developing intranuclear axoneme (Fig. 10). Following this insertion, the seven prominent giant mitochondria begin to aggregate at the base of the nucleus and eventually become closely associated with the developing flagellar axoneme (Fig. 11). At various points, numerous distinct electron dense granules appear between inner mitochondrial membranes; many cristae are present as well. Cisternae of endoplasmic reticulum are scattered in the cytoplasm with numerous free and attached ribosomes. The nucleus is flattened basally and somewhat rounded apically. Laterally or apically, a single prominent Golgi-complex forms transfer vesicles, presumably containing pro-acrosomal material. The presence of cisternae of endoplasmic reticulum adjacent to transfer vesicles, suggests their participation in the synthesis of pro-acrosomal secretions (Figs. 9,11). Several transfer vesicles apparently aggregate and then fuse together to form the pro-acrosome. At least two pro-acrosomes were noted in several of the differentiating spermatids (cup to post-cup stage). Presumably these pro-acrosomes form a larger pro-acrosome, which initially exhibits an electron dense central core (Fig. 12) and finally gives rise to the acrosomal component of the sperm. The residual Golgi body moves toward the developing mid-piece and continues to produce transfer vesicles, possibly to aid in the removal of superfluous cytoplasm from the mid-piece, during the elongation phase of spermiogenesis.

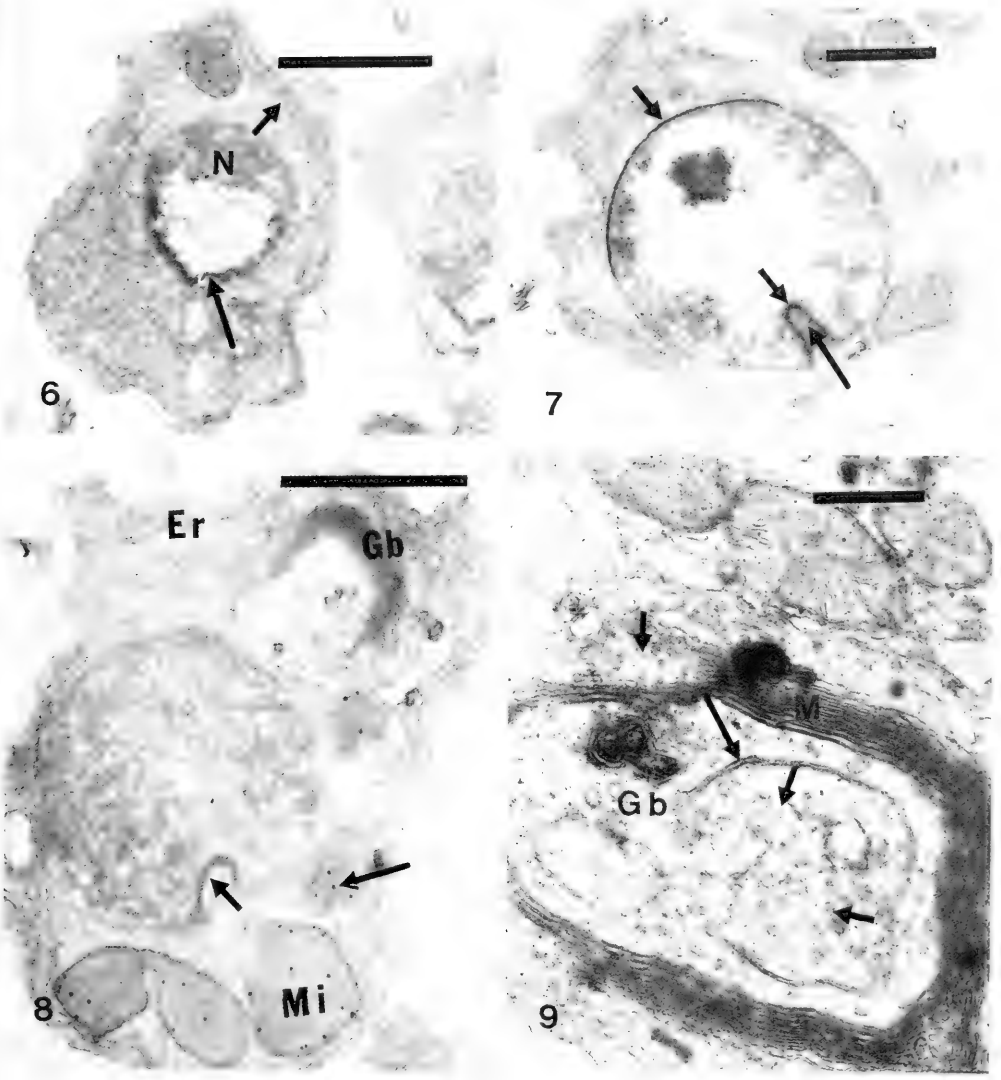
A proximal centriole was noted in some cells on the tip of developing intranuclear axonemes; while the distal centriole was seen posterior to the basal nuclear plate, and is



FIGS. 2–5. 2. Spermatogonium with large nucleus (N) and prominent nucleolus (arrow). Note scanty cytoplasm (C) with few cytoplasmic organelles. Bar = 4 μm. Figs. 3–5. Spermatocytes. 3. Spermatocytes showing patchy chromatin with or without nucleoli (long arrow). Golgi body (short arrow), mitochondria (Mi), acinar wall (Aw). Bar = 10 μm. 4. Secondary spermatocyte with well-formed Golgi body (Gb), cluster of mitochondria (Mi), nuclear membrane (arrow). Bar = 1.5 μm. 5. Late secondary spermatocytes joined by a cytoplasmic bridge (long arrow). Note basal-apical nuclear thickenings (short arrows). Nucleus (N). Bar = 2.5 μm.

closely associated with the cluster of giant mitochondria (Fig. 10).

Stage C (post-cup stage) spermatids are characterized by condensation of granular chromatin, fusion of giant mitochondria to form the Nebenkern around the flagellar axoneme, and further differentiation of the pro-acrosome into the acrosomal component of the sperm head. Depending on the stage of transformation, the nuclei are spherical with a flattened basal plate, ovoid or somewhat elongate. Along the antero-posterior axis of the nucleus, granular chromatin material condenses into fibrous strands, which fuse and form lamellar chromatin. Formation of lamellae commences peripher-



FIGS. 6–9. Stage A (Pre-cup) spermatids. 6. Basal thickenings of spermatids, showing the start of invagination (long arrow). Note chromatin condensation and less electron dense area basally. Nucleus (N), endoplasmic reticulum (short arrow). Bar = 2 μm. 7. Spermatid with apical and basal thickenings (short arrows). Developing intranuclear canal (long arrow). Bar = 1 μm. 8. Large mitochondria (Mi) with electron dense granules (long arrow), Golgi body (Gb), and numerous cisternae of endoplasmic reticulum (Er). Note chromatin condensation within nucleus and basal plate invagination (short arrow). Bar = 1.5 μm. 9. Stacked saccular membranes (M) of Golgi body (Gb), and endoplasmic reticulum (long arrow), with transfer vesicles (short arrows). Bar = 0.5 μm.

ally (Figs. 13, 14, 15, 16), and they appear in cross sections to adhere to the inner surface of the nuclear envelope (Fig. 15). Also, clustered giant mitochondria fuse, elongate, and form the Nebenkern (= mitochondrial sheath) enclosed within a common outer mitochondrial membrane (Fig. 16).

Stage D (elongate stage) spermatids further increase in length until they reach a typical filiform shape. The lamellar chromatin forms a single homogeneous mass of

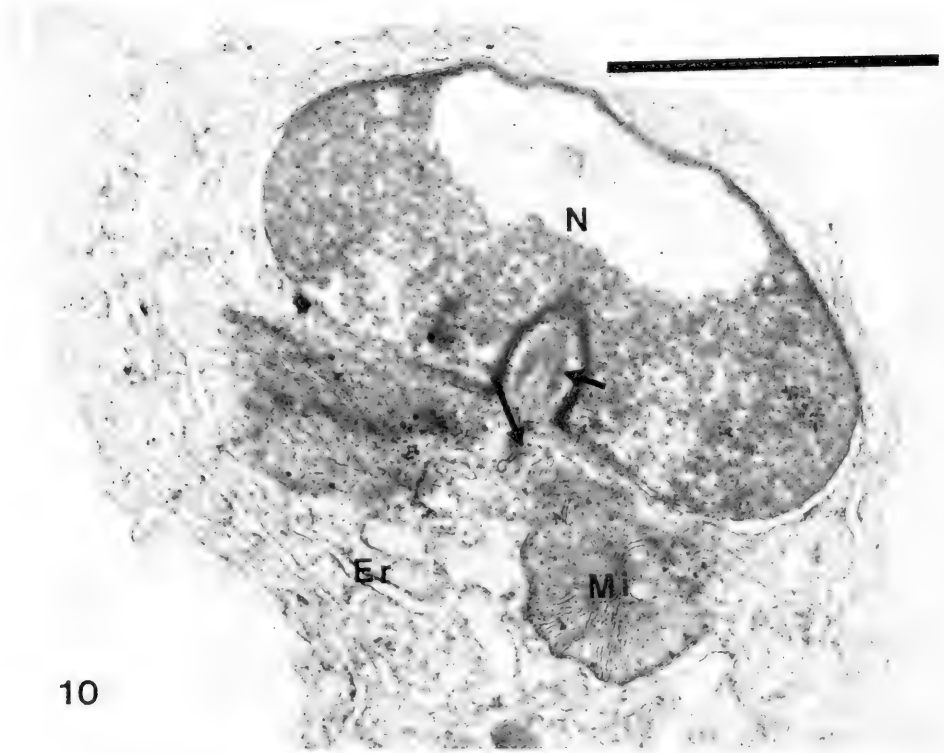
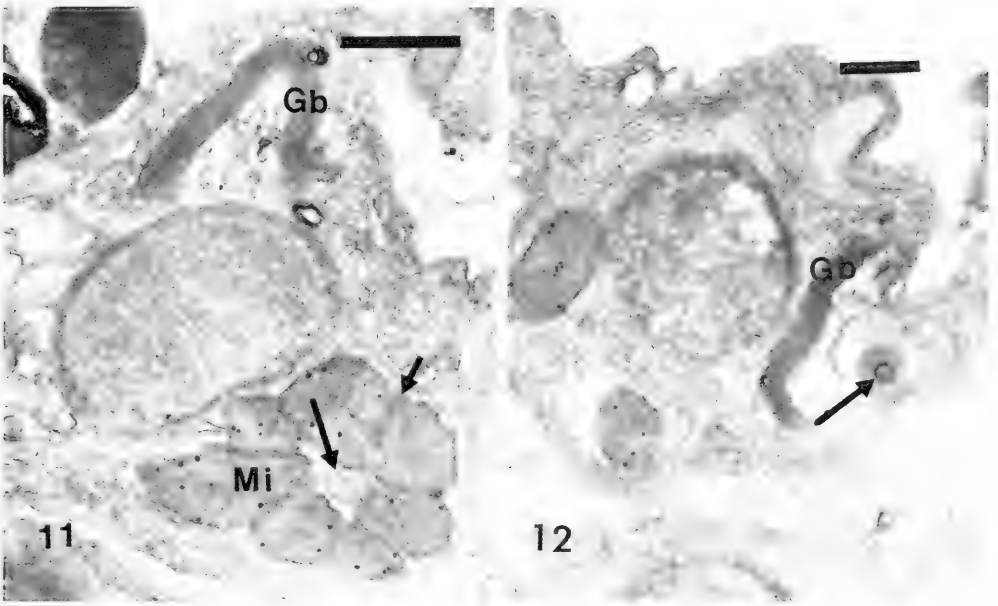


FIG. 10. Stage B (Cup-shaped) spermatid. Nucleus (N) showing developing intranuclear axoneme (short arrow). Distal centriole (long arrow), with microtubules posterior to intranuclear canal. Note granular chromatin material in nucleus. Mitochondria (Mi), endoplasmic reticulum with ribosomes (Er). Bar = 1.0 μm.

nucleoprotein that winds around the nuclear axoneme, giving the sperm head a helicoidal or corkscrew form (Figs. 17, 18). Likewise, the Nebenkern spirals around the flagellar axoneme (Figs. 17, 19), and has numerous prominent cristae (Fig. 20). Extrusion of residual cytoplasm in the head takes place anteriorly, as shown by the presence of cytoplasmic fragments lying close to the apex of sperm heads (Fig. 18). Extrusion of more superfluous cytoplasm also apparently takes place in the posterior end of the developing sperm, judging from the large cytoplasmic accumulation in the tail region (Figs. 18, 19). The sperm head and mid-piece are supported by an axoneme of typical cartwheel pattern of 9 + 2 microtubules (Figs. 13, 20). Stage D spermatids, characterized by homogeneous nucleoprotein and a Nebenkern undergoing elongation, also have a row of microtubules around the nucleus and mitochondrial sheath. Microtubules around the nucleus do not appose with the nuclear membrane (Fig. 21), while those around the Nebenkern lie side by side with the outer mitochondrial membrane (Fig. 22). These microtubules were not seen in earlier stages. In mature sperm, however, either very few scattered microtubules remained or they were absent (Figs. 23, 24).)

There are Sertoli cells closely associated with developing spermatocytes and differentiating spermatids and are confined to the acinar wall (Figs. 25, 26). Sertoli cells showed mid-pieces and nuclei of elongate spermatids embedded in their cytoplasm, as well as junctional complexes (= gap junctions) with spermatocytes (Fig. 26). Although there are a few biflagellated sperm, atypical forms such as the oligopyrene and apyrene types reported in many other prosobranch snails were not observed in *O. h. quadrasi*.

Mature sperm are long, with the mid-piece and tail comprising about 90% of their entire length. They possess a cone-shaped acrosome and a spirally twisted nucleus measuring 0.5–1.2 μm wide and 5.0–7.3 μm long (Figs. 25, 27).



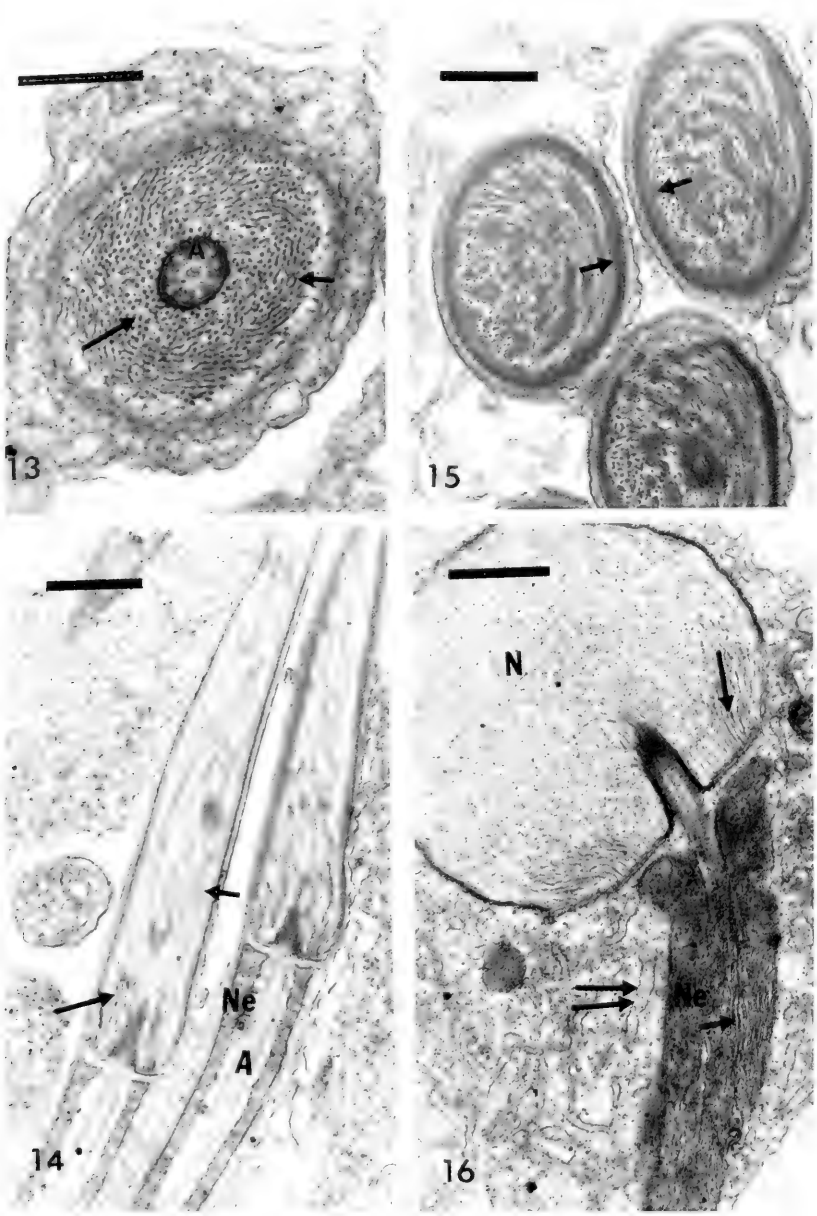
FIGS. 11, 12. Stage B (Cup-shaped) spermatids. 11. Spermatid with seven giant mitochondria (Mi), undergoing fusion, surrounding flagellar axoneme (long arrow). Note common, outer mitochondrial membrane (short arrow). Golgi body (Gb) with secretory granules. Bar = 1.0 μm. 12. Golgi body (Gb) adjacent to a pro-acrosome with electron dense central core (arrow). Bar = 1.0 μm.

DISCUSSION

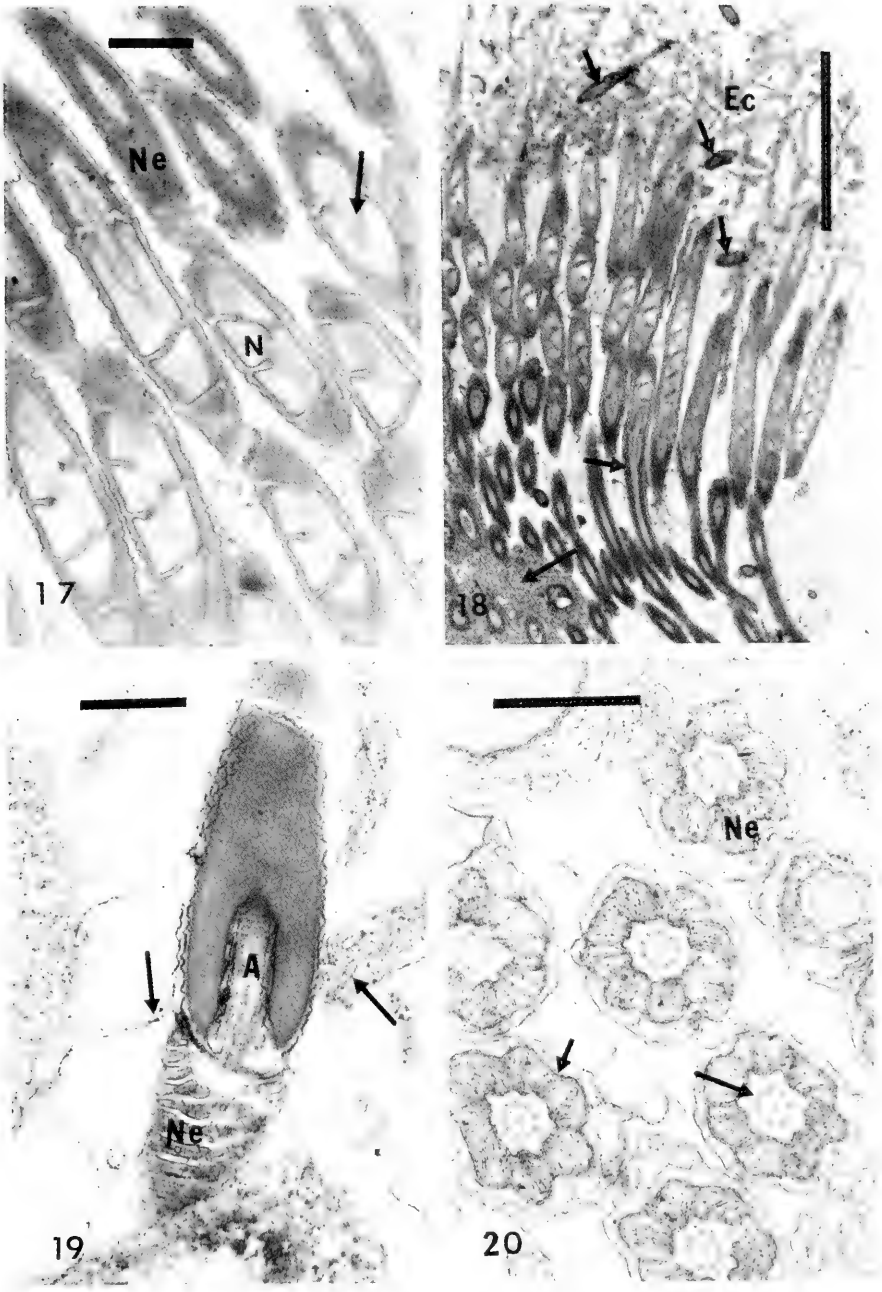
Among prosobranch snails, condensation of chromatin materials during spermiogenesis transforms the nucleus into a homogeneous mass of nucleoprotein. Nuclear aggregation of fibrous strands in *Littorina sitkana* (Buckland-Nicks & Chia, 1976) and *Ocenebra erinacea* (Féral, 1977) begins both at the center and on the periphery. In *Oncomelania h. quadrasi,* however, lamellar chromatin formation commences peripherally and moves inward to the center of the nucleus, in a pattern similar to that of *Cipangopaludina malleata* (Yasuzumi & Tanaka, 1958) and *Colus stimpsoni* (West, 1978).

The helical form of the sperm head of *O. h. quadrasi* resembles that of *Viviparus* spp. (Hanson *et al.,* 1952; Kaye, 1958; Gall, 1961), *C. malleata* (Yasuzumi & Tanaka, 1958), and *Truncatella subcylindrica* (Giusti & Mazzini, 1973). Interestingly, in *Nucella lapillus,* the head shaft initially forms a gentle spiral of 5–7 turns clockwise, with no corresponding twisting in the flagellar axoneme and the nucleus. As the sperm nucleus condenses and elongates to its final length, the head shaft is pulled out straight (Walker, 1970). Franzén (1970) noted a cytoplasmic spiral keel around the spirally-twisted nucleus and mitochondrial sheath, which enhances the corkscrew configuration of the nucleus of *Partulida spiralis,* a pyramidellid snail.

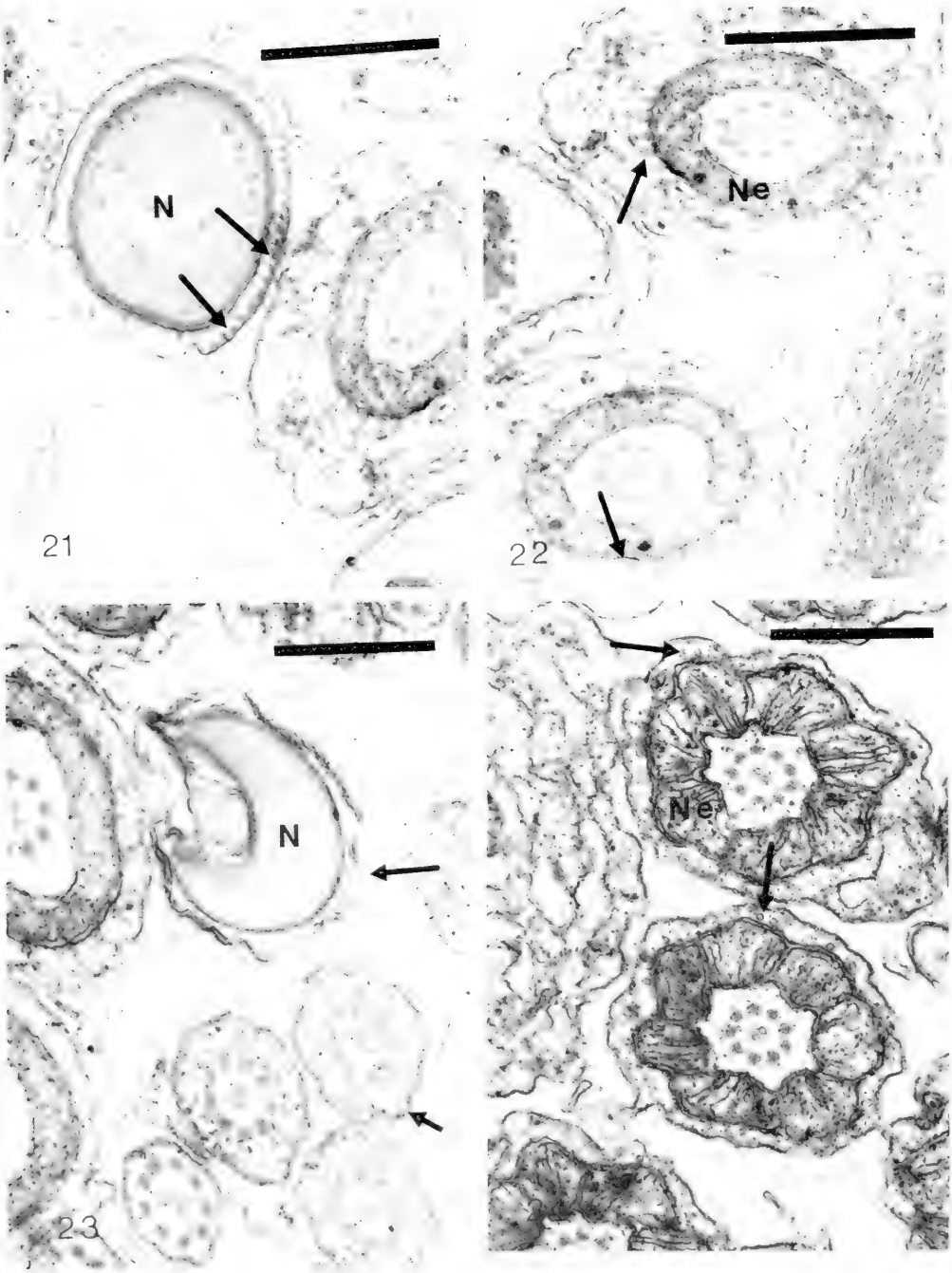
In *O. h. quadrasi,* a single row of microtubules (= manchette) was found around the nucleus and Nebenkern, similar to the arrangement in *Bithynia tentaculata* (Kohnert, 1980). In *C. stimpsoni* (West, 1978), the nucleus is helically wound with 3–5 rows of microtubules, biradially arranged and lying perpendicular to the two central axonemal fibers. Among snails with cylindrical sperm heads, Buckland-Nicks & Chia (1976), and Walker (1970) observed microtubules during later stages of condensation and suggested that these microtubules may assist in nuclear elongation and provide a sufficiently rigid support to sustain the shaping of the sperm head. Microtubules during later stages of nuclear condensation also were noted in *O. h. quadrasi* and apparently participate in the elongation process. However, the role these microtubules play in determining the helicoidal shape of the sperm head is doubtful, judging from the location of the microtubules relative to the nuclear mem-



FIGS. 13–16. Stage C (Post-cup) spermatids. 13. Cross section of spermatid nucleus showing intranuclear axoneme (A) and partially condensed chromatin. Note fibrous (long arrow) and lamellar chromatin (short arrow) peripherally. Bar 0.5 μm. 14. Longitudinal section of spermatids, showing fibrous strands of chromatin (long arrow) chromatin lamellae (short arrow). Nebenkern (Ne) around flagellar axoneme (A). Bar = 1.0 μm. 15. Cross sections of nuclei. Advanced stage of chromatin condensation with less fibrous chromatin. Note thick chromatin lamellae on the inner surface of nuclear envelope (short arrows). Bar = 0.5 μm. 16. Longitudinal section of early stage C spermatid, with granular chromatin forming fibrous strands (long arrow). Note development and elongation of Nebenkern (Ne) and distinct cristae (short arrow). Endoplasmic reticulum with ribosomes (double arrows). Bar = 1.0 μm.



FIGS. 17–20. Stage D (elongate) spermatids. 17. Tangential sections of corkscrew-shaped nuclei (N) and Nebenkern (Ne) spiralled around axoneme (arrow). Bar = 1.0 μm. 18. Early stage elongate spermatids with extruded cytoplasm (Ec) apically. Note superfluous cytoplasm (long arrow) in sections of mid-piece (short arrows). Bar = 6.0 μm. 19. Tangential section of spermatid with fragments of residual cytoplasm (arrows) attached to plasma membrane. Note spiral Nebenkern (Ne) and nucleus supported by axoneme (A). Bar = 0.5 μm. 20. Cross sections of mid-piece. Flagellar axoneme (long arrow). Seven mitochondria composing the Nebenkern (Ne) are evident. Note common outer mitochondrial membrane (short arrow) and numerous cristae. Bar = 1.0 μm.

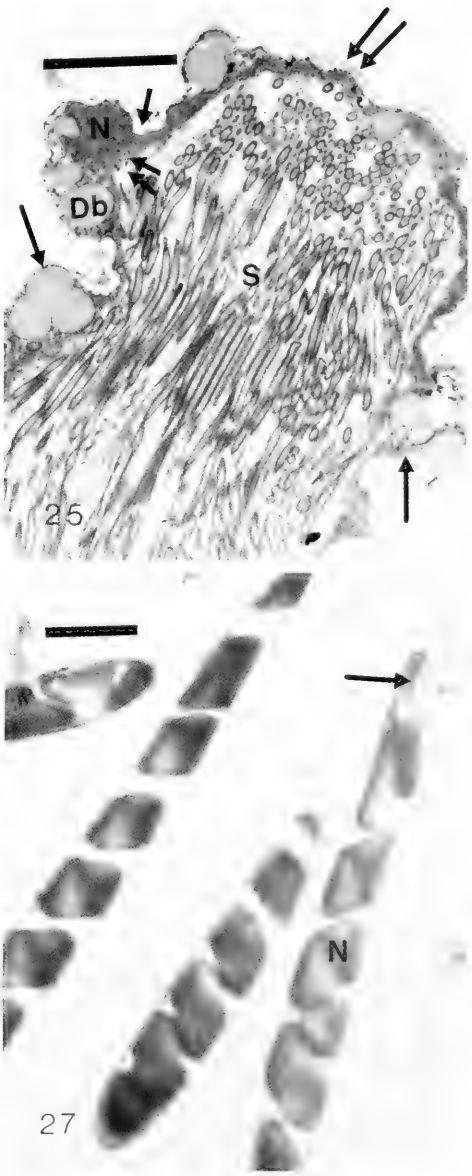


FIGS. 21–24. 21. Cross section of spermatid with microtubules (arrows) around nucleus (N). Note distance of microtubules from nuclear membrane. Bar = 0.5 μm. 22. Cross sections of mid-piece of spermatids with row of microtubules (arrows) beside outer membrane of Nebenkern (Ne). Bar = 0.5 μm. 23. Cross section of sperm head with helicoidal nucleus (N). Plasma membrane (long arrow), sections of end-piece of sperm tail (short arrow). Bar = 0.5 μm. 24. Cross sections of mid-piece of elongate spermatids. Note widely scattered microtubules (long arrows) and shape of Nebenkerne. Bar = 0.5 μm.

brane (Fig. 21), and therefore requires further investigation. Fawcett *et al.* (1971) found that in avian finch sperm, which have a rather complex corkscrew-shaped sperm head, microtubules are absent during the elongation process. During the intermediate and late stages of differentiation, when the helical form of the nucleus is already evident, 6–8 rows of microtubules are associated with the nucleus. They postulated that microtubules are probably not essential in initiating the helical shape of the nucleus. Most of their evidence favors the view that the configuration of the finch sperm head is determined by intrinsic nuclear factors, and not by the helical microtubules of the manchette. They further argued that microtubules, extending posteriorly and spiralling around the Nebenkern, form a temporary organelle homologous to the manchette, which induces spermatid elongation and may even determine the form of the mitochondrial sheath. In *O. h. quadrasi,* microtubules around the Nebenkern are closely apposed to the outer mitochondrial membrane (Fig. 22), suggesting a similar function. While the Golgi body makes no apparent contribution to the formation of acrosome in *Nerita senegalensis* (Garreau de Loubresse, 1971), in *O. h. quadrasi,* a single Golgi body is involved in acrosome formation.

Morphologically and physiologically, giant mitochondria that compose the Nebenkern vary extensively among different phyla and species (Anderson & Personne, 1976; Franzén, 1970). The pattern of Nebenkern formation even varies to a certain degree among prosobranch species. *Cipangopaludina* spp. (Yasuzumi & Tanaka, 1958; Yamasaki, 1966), *O. erinacea* (Féral, 1977) and *C. stimpsoni* (West, 1978) have two giant mitochondria. In *Cipangopaludina* spp. these mitochondria are tightly spiralled around the flagellar shaft as separate bodies. There are 4–5 giant mitochondria in *Littorina sitkana* (Buckland-Nicks & Chia, 1976). *N. lapillus* (Walker, 1970) and *Viviparus contectoides* (Kaye, 1958), 7–9 in *L. lambis* (Koike & Nishiwaki, 1980) and 9 in *B. tentaculata* (Kohnert, 1980). In *O. h. quadrasi* there are usually 7 giant mitochondria enclosed within a common outer mitochondrial membrane, with their inner membranes intact.

It seems rather unlikely that the Sertoli cells of *O. h. quadrasi* play an active role in the transportation of spermatogenic cells. Such conjecture is based on the location of Sertoli cells, which is on the periphery of the acinar



FIGS. 25, 27. 25. Section of testicular acinus packed with sperm (S). Note Sertoli cells (long arrows) and germinal epithelium (two long arrows), with a less electron dense homogeneous layer (short arrow). Mid-piece partly embedded in a Sertoli cell (two short arrows). Nucleus (N), electron dense bodies (Db). Bar = 10 μm. 27. Longitudinal sections of sperm heads showing cone-shaped acrosome (arrow) and helicoid nucleus (N). Bar = 1.0 μm.

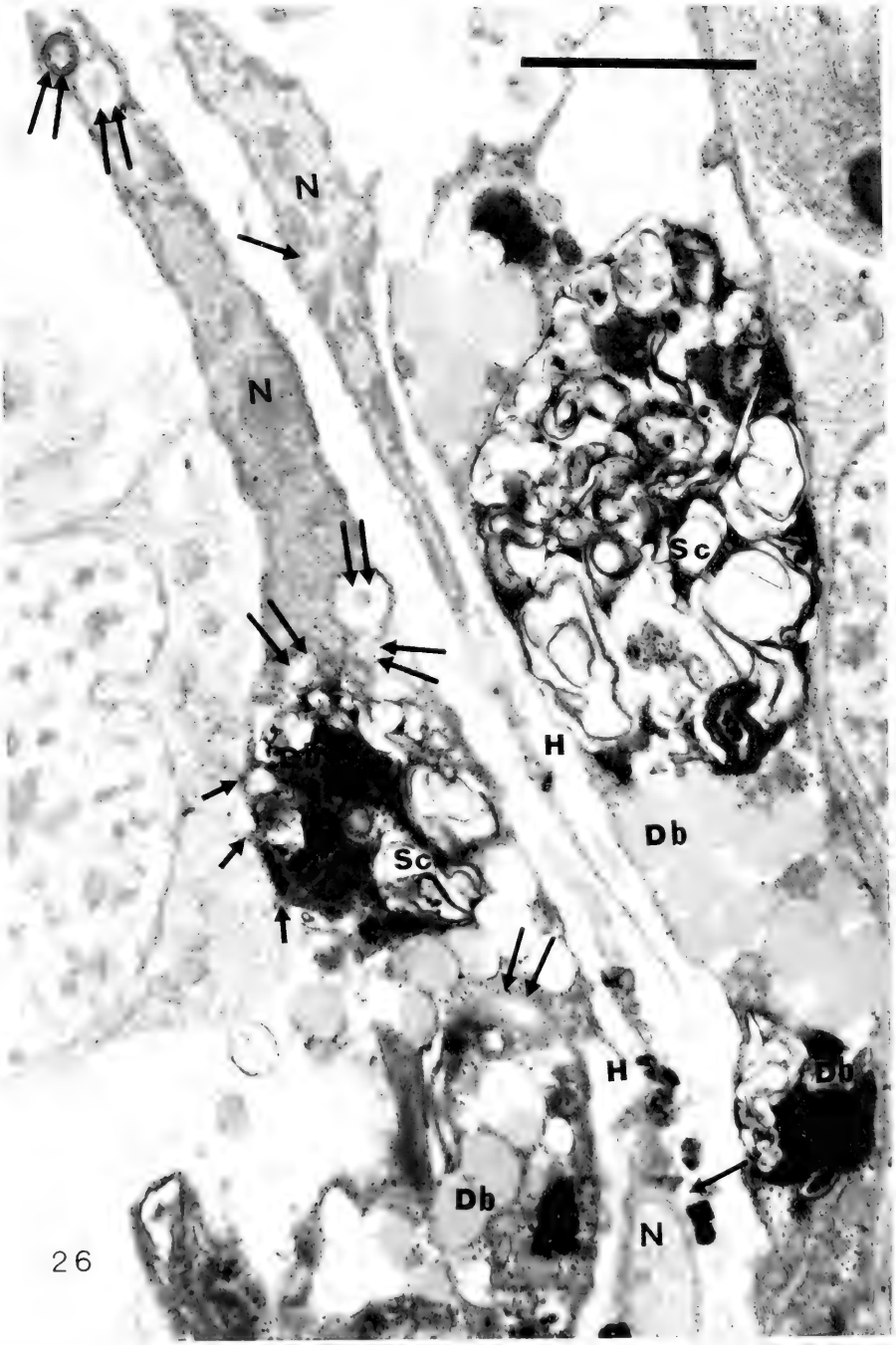


FIG. 26. Portion of acinar wall of two juxtaposed testicular acini. Each acinar wall has an inner layer of Sertoli cells (Sc), a middle homogeneous layer (H) and an outer germinal epithelium (long arrows). Note electron dense bodies (Db) and other cytoplasmic inclusions within Sertoli cells, several sections of mid-piece and nucleus of elongate spermatids embedded in the cytoplasm (two long arrows). Also, note desmosome-like processes (= gap-junctions) (short arrows) between a spermatocyte and a Sertoli cell. Nucleus (N). Bar = 3.0 μm.

wall. Also, the absence of defined cytoplasmic microfilaments in association with sperm are not present. There are indications, however, that Sertoli cells are involved in the nutrition of developing spermatocytes and differentiating sperm, evidenced by the presence of some junctional complexes with spermatocytes and elongate spermatids, embedded in their cytoplasm (Fig. 26). Some electron dense cytoplasmic inclusions in Sertoli cells resemble residual bodies reported in *Biomphalaria glabrata* (De Jong-Brink *et al.,* 1977), which suggest that cells possibly function in phagocytosis of superfluous, extruded cytoplasm.

Sperm dimorphism, that is the production of typical and atypical sperm has been reported in many prosobranch snails (Nishiwaki, 1964; Tochimoto, 1967; Koike & Nishiwaki, 1980). In *O. h. quadrasi,* although a few biflagellated sperm were observed, they do not resemble the atypical apyrene and oligopyrene sperm reported in other prosobranchs. Physiological dimorphism is possible in *O. h. quadrasi,* a form of dimorphism suggested to occur in *C. stimpsoni,* which have only typical eupyrene sperm.

ACKNOWLEDGMENTS

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THE INITIAL STAGES OF RADULAR DEVELOPMENT IN CHITONS (MOLLUSCA: POLYPLACOPHORA)

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ABSTRACT

The initial stages of development of the chiton radula were examined in *Mopalia lignosa* (Gould, 1846), *M. muscosa* (Gould, 1846), *Lepidochitona fernaldi* Eernisse, 1986, and *L. caverna* Eernisse, 1986. It starts in postmetamorphic juveniles with the secretion of the 2nd, 5th and 8th pairs of laterals, which are the main functional teeth of adult chitons. Moreover, it appears that juveniles are equipped with an efficient feeding instrument nearly as soon as radula formation begins, and certainly before the chitons have their complete set of teeth. This is evident from the mineralization of the 2nd laterals ("magnetite" teeth) from the start, the indications of normal degradation of "used" radula teeth in young juveniles, and observations of feeding in juveniles. As juveniles mature, new laterals are added between existing ones. The 1st laterals and the central tooth originate by fragmentation of a medial "precursor" plate. The phylogenetic implications of the polyplacophoran mode of tooth pattern formation are discussed and related to inferences concerning a primitive ancestral molluscan radula.

Key words: radula; Polyplacophora; chiton; morphogenesis; ontogeny; phylogeny.

INTRODUCTION

Comparative ontogenetic investigations of the molluscan radula have potential to reveal shared patterns of radular formation or divergent patterns that distinguish between particular lineages of mollusks. For all mollusks, only polyplacophorans (Minichev and Sirenko, 1974; French translation by Sirenko & Minichev, 1975), Solenogastres ("aplacophorans") (Salvini-Plawen, 1972, 1978), and pulmonates (Kerth, 1979) have been thoroughly investigated. Minichev and Sirenko (1974) describe the radula in several genera of "larval" polyplacophorans as having a broad, monostichous form. They conclude from this observation that the radula of primitive mollusks is derived from a monostichous ancestral state. Salvini-Plawen (1981; 1985) has reached similar conclusions for two species of Solenogastres (or Neomeniomorpha), based on Pruvot's famous larva (Pruvot, 1890) and his own observations (Salvini-Plawen, 1972, 1978) of *Simrothiella*, although he shows only a slender connection between two already well shaped halves in *Simrothiella.* In contrast, Kerth (1979) showed that the radulae of embryos of several pulmonate families pass through a distichous stage.

If the general scheme proposed by Minichev and Sirenko (1974) and Salvini-Plawen (1985) is correct, then polyplacophorans (also referred to as chitons hereafter) and "aplacophorans" would appear to have a fundamentally different ontogenetic sequence of radular development from pulmonates, suggesting a possible phylogenetic discontinuity. This, and the availability of chiton larvae, led us to reexamine the process of radular formation in chitons. Here we reexamine the ontogeny of radular development in four chiton species: *Mopalia lignosa*, *M. muscosa*, *Lepidochitona fernaldi*, and *L. caverna.* The successful culturing of chitons through metamorphosis has been difficult for most workers, and often published descriptions have been based on cultures with a low percentage of metamorphosing juveniles. These four species were selected because of the fortuitous availability of healthy larvae and juveniles. In retrospect, this selection also permitted comparisons between two families, between closely related species of two genera, and between free spawners (both *Mopalia* spp.) and brooders (both *Lepidochitona* spp.). Finally, we infer a more general view of the basic polyplacophoran radula from our comparisons of these four species, and compare this view to the one proposed by Minichev & Sirenko (1974).

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MATERIALS AND METHODS

Larvae and juveniles of four chiton species were obtained from adult chitons spawning at Friday Harbor Laboratories. All adults except for *Lepidochitona caverna* were collected on San Juan Island, Washington U.S.A. Adults of *L. caverna* were descended from a breeding population first maintained at Santa Cruz, California U.S.A. (site of original collection, Eernisse, 1984; 1986) and later at Friday Harbor Laboratories on San Juan Island.

Mopalia lignosa and *M. muscosa* free spawned their gametes, and embryos hatched as swimming larvae in less than two days after fertilization for *M. lignosa*, and less than four days after fertilization for *M. muscosa*. These swimming larvae were maintained in beakers for approximately one week with daily changes of filtered sea water and kept at ambient sea water temperatures (12 to 14° C). For each of the following samples of known age, the radulae of 4 to 14 specimens were examined, all fixed in 70% ethanol.

The first series of larvae and juveniles was obtained from a single spawning *M. muscosa* female and several lightly spawning males, on June 10, 1985. Fixations were made at 7, 8, 9, 10, 11, 13, 15, 17, and 21 days after fertilization. Less than 10% of several hundred larvae had metamorphosed by 13 days, when a selection of representative larvae and all the benthic survivors of one of three cultures were fixed. At 17 days, about 40% of the remaining larvae in the cultures had metamorphosed and the benthic survivors of the better of the two remaining cultures were fixed. Finally, at 21 days, nine metamorphosed juveniles were fixed.

The best series of larvae and juveniles was obtained from two *M. lignosa* spawning within hours of collection on August 1, 1985. An isolated male spawned first, and his sperm were introduced to an isolated female, prompting her to spawn copiously. The resulting larvae were observed at least daily until they were near to metamorphosis. The first fixation was made at eight days, when about 40% of the larvae in all cultures (and in the fixed subsample) were metamorphosed. The next fixation was made at 12 days, approximately one day after more than 95% of the larvae had completed metamorphosis. Subsequent fixations for this study were made at 18, 22, 29, 36, 51, 66, and 105 days after fertilization (length of juveniles examined: 0.35 to 1.6 mm), and other animals from this cohort were kept alive including 19 that were still alive at 14 months (mean length ± st. dev. = 21.4 mm ± 3.26; max. length = 27.6 mm; min. length = 13.3 mm).

In contrast to the free spawners *M. muscosa* and *M. lignosa*, the brooders *L. fernaldi* and *L. caverna* care for their embryos until the emerging larvae are capable of crawling and are within one or two days of metamorphosis (Eernisse, 1984). A large selection of adult brooders of these species were kept in the lab, and juveniles were collected near adults shortly before or after they had metamorphosed. For *L. caverna*, these juveniles ranged from recently metamorphosed, about 0.5 mm length, to considerably older juveniles, to a maximum of 1.8 mm length. For *L. fernaldi*, we examined a series of juveniles ranging from 1.1 mm to 1.6 mm length. The exact age of juveniles collected in this way could not be determined. However, for *L. fernaldi*, additional broods were removed from three adults and cultured as for *M. lignosa* and *M. muscosa*. The age of each of these three broods (i.e. days since fertilization) was estimated with a high degree of confidence based on the appearance of previously timed developmental features in the embryos (Eernisse, 1984). Their age in relation to metamorphosis could be determined by direct observation. All 45 larvae and juveniles from one brooder were fixed on July 11, 1985, when about 70% of the larvae had metamorphosed (approx. 13 days after fertilization), including 1 of 45 metamorphosed on July 8 (at 10 days), and 15 of 45 on July 10 (at 12 days). Juveniles from two other brooders were fixed at about 19, 25, and 28 days after fertilization. The length of the 13 to 28 day old *L. fernaldi* juveniles ranged from 0.35 mm to 0.5 mm.

In addition to the above species, we examined premetamorphic larvae of *Lepidochitona cinerea* (Linnaeus, 1767) (a kind gift from Prof. Dr. W. Haas, Bonn, Fed. Rep. of Germany).

In preparation for phase contrast and Nomarski-interference contrast light microscopy, specimens were first rehydrated, then the calcareous dorsal plates and girdle spicules were dissolved with 1N HCl. Next, specimens were macerated in cold 5-10% KOH (1 to 2 h). Finally, the radulae were prepared by pressing the macerated tissue under a cover glass in hot glycerine gelatine.

For SEM observations, juveniles and adults were macerated in warm 5% KOH only until

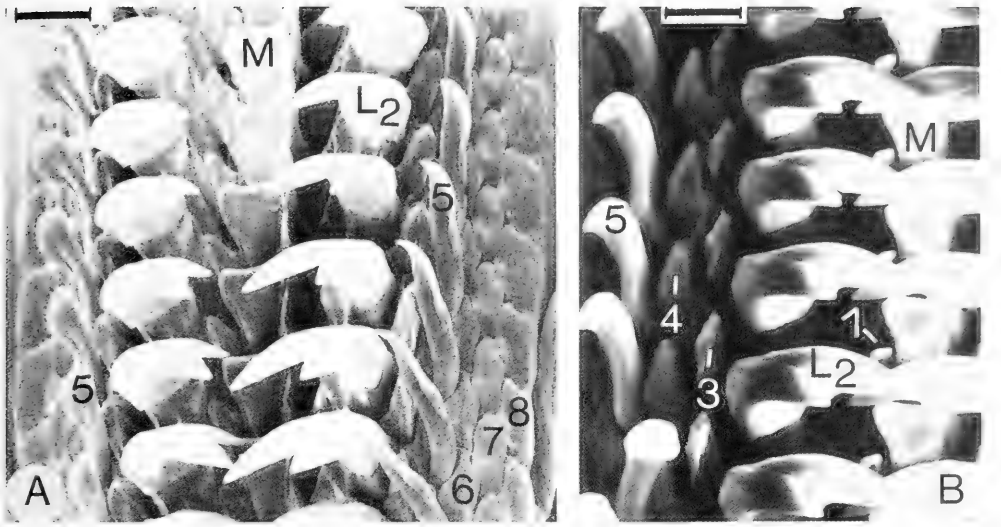

FIG. 1: Radulae of adult *Mopalia lignosa* in dorsal (slightly lateral) views with SEM. A. Adult (length 43 mm) from San Juan Is., Washington, USA; total radula length 8.6 mm with 33 transverse rows of mineralized teeth. Complete transverse rows 6-11, scale bar: 190 μm. B. Small adult (length 16 mm) from Año Nuevo Pt., California, USA. Left central portion of transverse rows (approx. ⅓ distance from first anterior row) with teeth spread in preparation, scale bar: 80 μm. L_1 to L_8 = laterals, M = medial (central) tooth.

the radulae were clean and could be teased away from other tissue. After a distilled water rinse, radulae were stored in 70% ethanol, then transferred to 100% ethanol before mounting on a specimen stub. The radulae were sputtercoated with gold for two to six minutes and scanned at 40 kV on a JEM 1200 EX™ STEM (in conjunction with energy dispersive Xray microanalysis of juvenile and adult radulae as reported in a subsequent study, Eernisse and Fontaine, in prep.) or at 15 kV on a JEOL SM-35 SEM.

RESULTS

The chiton radula

The chiton radula is remarkably uniform in tooth number and type, bearing transverse rows of 17 teeth of predictable shapes (Figs. 1A,B) except 11 or 13 teeth per row in *Juvenichiton* (Sirenko, 1975). Each row is "stepped," or v-shaped, with each tooth anterior (at its base) to the next most distal tooth. The eight lateral teeth (L_1 to L_8) on each side of the medial or "central" tooth ("M") are attached to the elastic radular membrane. The L_2 and L_5 pairs are the most elongate teeth. The L_2 pair are the main working teeth and bear highly magnetized dark caps (Lowenstam, 1962; Carefoot, 1965; Towe and Lowenstam, 1967), usually each with one to three sharp cusps. Each L_5 tooth has the general appearance of a sickle, usually with a flattened distal tip, and lies in close association over the mineralized portion of the L_2 tooth from one row posterior. The relationship of the L_5 and L_2 cusps suggests that they cooperate in scraping and collecting food or, alternatively, the L_5 cusps protect other soft parts from the highly mineralized L_2 cusps as these teeth roll back into their normal tube-like orientation. Finally, the margins of the radula are stabilized by the plate-like $L_{6,7,8}$.

The development of the juvenile radula

We found no radular structure in "trochophore" larvae (those larvae that still had a prototroch); the radula first appears after metamorphosis. Even the specimens of *L. cinerea* with conspicuous valve rudiments (plate-anlagen) lacked radulae. The first radulae were recognized in *M. lignosa* 8 days after fertilization (3 to 6 longitudinally repeated, transverse rows of teeth); in *M. muscosa* in the course of the first week after metamorphosis (up to 10 transverse rows);

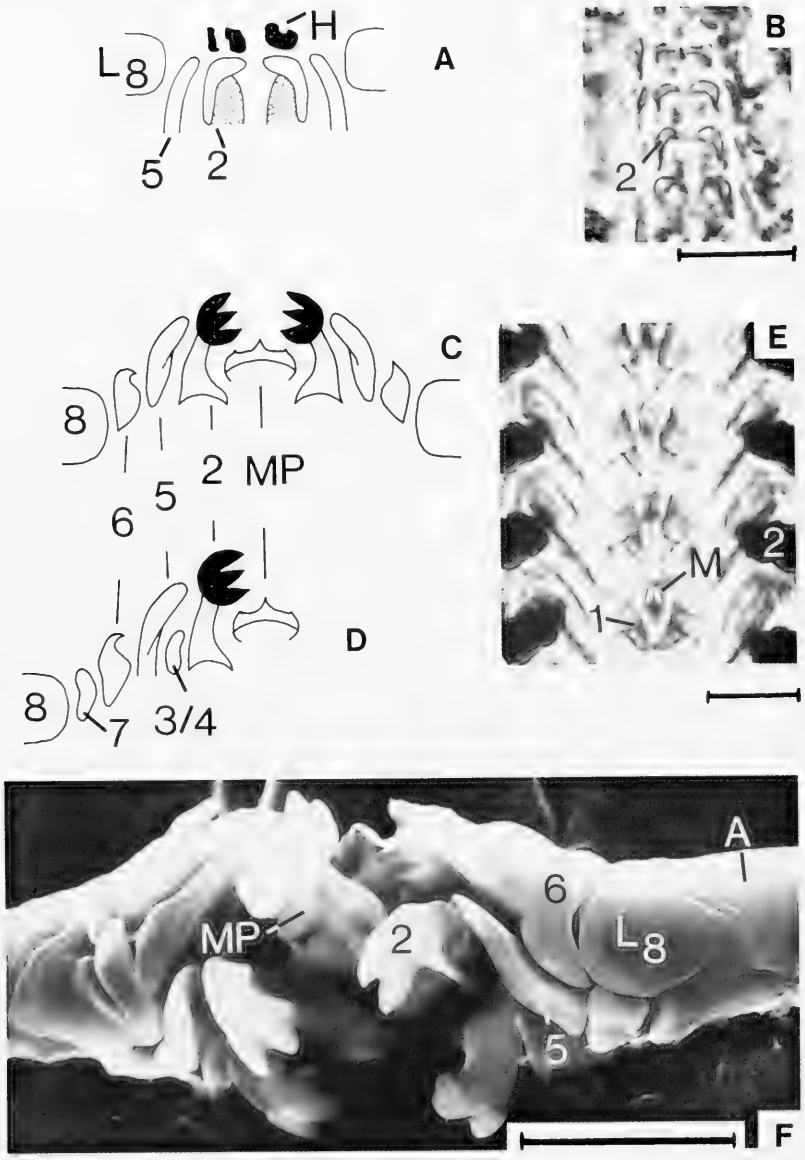

FIG. 2: Radula morphogenesis in juvenile chitons. A. Foremost transverse row of the larval radula with 3 pairs of teeth (composite reconstruction with teeth slightly separated from camera lucida drawings of *Mopalia lignosa, M. muscosa, Lepidochitona fernaldi*). H = hump. B. Earliest larval radula of *L. fernaldi* 13 days after fertilization, phase contrast, scale bar: 20 μm. C. Transverse row with 9 teeth (composite reconstruction as in Fig. 2A of *M. lignosa, M. muscosa, L. fernaldi*) corresponding with Fig. 2F. D. Transverse row with 13 teeth (camera lucida drawing as in Figs. 2A,C of *L. fernaldi*). E. L_1-pair and medial tooth (*L. fernaldi* oldest series). Compare with the medial "precursor" plate (MP) in younger juveniles (Figs. 2 C,F), phase contrast, scale bar: 20 μm. F. Bending plane of the radula, anteroventral SEM view (*M. lignosa*, 19 days after fertilization). Note the shape of the medial plate. Scale bar: 10 μm, A = alar membrane (subradular membrane) of the radula.

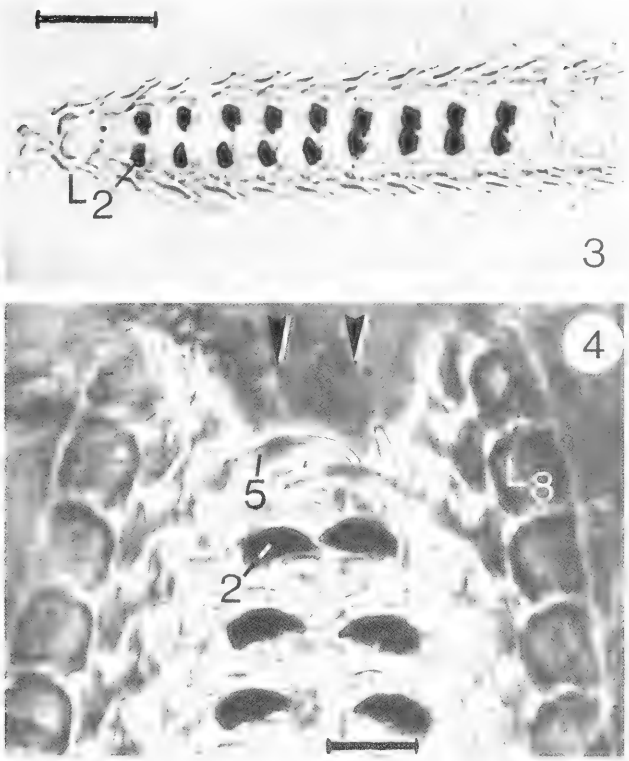

FIG. 3: Light micrograph dorsal view of radula (*L. fernaldi* 25 days after fertilization) with prominent dark-capped cusps ("magnetite teeth") of L_2-pairs. Left to right: anterior to posterior. Scale bar: 20 μm.
FIG. 4: Anterior end of a juvenile (1.1 to 1.6 mm length) radula with degradation. The foremost inner laterals and the medial tooth are shed (arrows). (*L. fernaldi*, phase contrast). Scale bar: 20 μm.

and in *L. fernaldi* 13 days after fertilization (4 to 7 transverse rows).

The tooth shape and pattern were identical in the youngest specimens of *M. lignosa*, *M. muscosa*, and *L. fernaldi*: Radular development consistently starts simultaneously with the paired formation of the "magnetite teeth" (L_2), the sickle-shaped L_5 teeth, and the outermost marginal plates (L_8). Therefore the first transverse row of the newly formed radula usually consists of 6 teeth (pairs of $L_{2,5,8}$). More rarely 4 teeth (pairs of $L_{2,5}$) or, much more rarely, a single pair (L_2) was noted. Each tooth was easily identified from the start by its characteristic shape (Figs. 2A,B,F). There is a bilaterally-symmetrical tooth pattern from the outset. The cusps of the anterior-most L_2 pair were dark-capped even in the earliest cases, suggesting mineralization of these cusps from the onset of radular formation. Moreover, juveniles of all species considered here began active foraging movements within a week of metamorphosis, moving from side to side and leaving a trail of corresponding rasp markings in the substrate covering of diatoms. A medial tooth could not be detected in early stages of radular development. This finding is contradictory to observations of *Schizoplax* by Sirenko & Minichev (1975: figs. 2b,c). In a few cases amorphous humps ("H" in Fig. 2A) were observed in front of the foremost plates.

Further radular development was documented in *M. lignosa*, *L. fernaldi* and *L. caverna*. The radula elongates and the number of transverse rows increases up to 30 to 40 in the oldest series. Several of the 18 to 28 day old juveniles showed radula degradation which is characteristic of all older animals. For juveniles of all ages as in adults, the medial part of the radula's anterior end was shed first (Fig. 4).

New longitudinal rows of teeth appear with the increasing age of the chitons. A large

medial plate ("MP," Figs. 2C,D) and the L_6 teeth are next to be added between the existing $L_{2,5,8}$ teeth. The radular development is identical in the three species until each has nine teeth within the transverse row, but differences were observed after this stage. In each transverse row, additional teeth form in the order L_7, L_4 and L_1 in *L. fernaldi* and *M. lignosa*, but L_4, L_7 and L_1 in *L. caverna*. Almost all of the oldest specimens of *Lepidochitona* appeared to exhibit a complete radula with 17 teeth in the transverse row, although the small L_3 could not be identified unequivocally. New teeth are added to the radula of *M. lignosa* very slowly by comparison. The oldest juveniles have at most 11 longitudinal rows of teeth in their radula.

Minichev & Sirenko (1974) state that nearly all new teeth in the transverse row originate from a fragmentation of "precursor"-plates. Such a process can be excluded at least for the $L_{2,4,5,6,8}$ because we never observed these tooth pairs in an intermediate stage of fragmentation. On the other hand the medial plate apparently splits up to form the L_1 pair and the definitive medial tooth (Fig. 2D,H). This process was evident by comparing juveniles that had only a broad medial plate with juveniles at a slightly more advanced stage that had a small medial tooth flanked by the L_1 pair. The medial tooth could be identified as a small medial ridge on the "precursor"-plate before its apparent separation.

DISCUSSION

In the three species examined at early stages, radula formation starts soon after metamorphosis. First a symmetrical tooth pattern arises, consisting normally of several transverse rows of "teeth," each with three "tooth" pairs. A medial plate for each transverse row is added later. This sequence is basically the same as has been observed in radulae of many gastropods (Kerth, 1979; 1983a, b) including seven families of pulmonates and in two genera of opisthobranchs, *Polycera* and *Adalaria*, which pass through a stage with one to three pairs of laterals in each transverse row before a central tooth is added.

The radulae of the chitons we examined, however, differ considerably from those of the gastropods examined in their later development. In gastropods, new longitudinal rows of laterals are added only on the outermost margins of the radula (Kerth and Hänsch, 1977). In chitons, new longitudinal rows of laterals are inserted (i.e. erupt) between existing laterals. We found that the first teeth or plates to appear in a chiton radula are, appropriately enough, the main adult working $L_{2,5}$ teeth and L_8 plates, the latter previously suggested to serve as margin stabilizers. Evidence presented here would indicate that particular radular teeth are formed with characteristic shapes making them functional almost from the start, and certainly before all 17 teeth per row are present. Judging from their dark color, we concluded that the cusps of the initial L_2 pairs were apparently mineralized from the start. This result has more recently been confirmed with energy dispersive Xray microanalysis of *M. lignosa* juveniles only 16 days post-fertilization (Eernisse and Fontaine, in prep.). Finally, our observations of feeding behavior in newly metamorphosed juveniles provide strong evidence of the functionality of the newly formed radula.

Minichev and Sirenko (1974) described the radular development in four chiton genera and in some unidentified chiton "trochophores." Our results differ from theirs in several ways: (1) We observed no radulae earlier than postmetamorphic stages. (2) These authors describe a primordial radula in the unidentified trochophores with only one longitudinal row of broad plates. We didn't observe any comparable structure, although there occasionally were a few amorphous humps in front of the foremost transverse row (Fig. 2A). (3) Minichev and Sirenko (1974) depicted primary central teeth in the foremost parts of the youngest radula, but secretion of these teeth stopped very early. We were not able to find any comparable structure even with phase contrast or Nomarski-interference optics. (4) According to these authors, almost all of the laterals originate by fragmentation of a pair of "precursor"-plates on either side of the L_2 pair. Although the order of fragmentation is never explicitly stated in Minichev and Sirenko (1974: 1136), Sirenko and Minichev (1975: fig. 2b,c,d) clearly indicate that they believe the first fragmentation of the "precursor"-plates will lead to the adult L_3 and $L_{4\text{-}8}$ pairs, the next fragmentation to the L_4 and $L_{5\text{-}8}$ pairs, and so on until finally the L_7 and L_8 fragment. We can rule out such a fragmentation process in the species we examined for all laterals with exception of the L_1-pair. These and the medial tooth originate by fragmentation of the medial plate (Fig. 2C,E,F). (5) We observed a different order that new

teeth are added in each transverse row. Sirenko and Minichev (1975: figs. 2b,c,d) depicted very exactly the shape of the laterals in the earliest radula and because their drawings are completely in accordance with the shape of laterals in our investigated species, it is clear they have misinterpreted several teeth or plates. For example, the teeth or plates Sirenko and Minichev (1975: fig. 2d) have labeled L_3, L_4, and $L_{5\text{-}8}$ should instead be labeled L_5, L_7 and L_8, respectively. (6) Minichev and Sirenko (1974: 1136, fig. 1:4) argued that the dark portions of the L_2 pairs are a secondary feature, with the first few L_2 pairs lacking mineralization altogether. We observed mineralization concurrent with the start of radular formation.

There is a possibility that our results differ from those of Minichev and Sirenko (1974) because they studied different chiton species, or because some of their species differ because they are brooders (e.g. *Schizoplax brandtii* and *Hanleyella asiatica*). However, our consistent results for members of two chiton families, and for both free spawners and brooders, suggests to us that the patterns we have observed are general for chitons. If we are correct then our results are important not only in documenting a previously unknown pattern of tooth formation but are also important to recent discussions of molluscan evolution. This is true because the ontogeny of the chiton radula has been used as a prime case in favor of a bilateral yet monostichous ancestral condition. In order to appreciate both the underlying assumptions and previously stated support for this idea, some review is necessary.

The discovery of living monoplacophorans and descriptions of their anatomy (Lemche and Wingstrand, 1959; Wingstrand, 1985) has again brought to prominence the often suggested hypothesis that metamerism is a basic feature of mollusks, perhaps a primitive condition shared with other metameric protostomium ancestors. Organs are also repeated in polyplacophorans (chitons) and in the cephalopod genus *Nautilus* as was discussed in depth by Naef (1926) and previous authors (for review see Wingstrand, 1985). Particularly striking are the repetition of kidneys, atria and gills in monoplacophorans, polyplacophorans, and in *Nautilus*. Other authors regarded the metameric condition as a convergence (Hoffmann, 1937; Boettger, 1959; Yonge, 1960; Salvini-Plawen, 1985) and argued that single paired systems were present in a hypothetical molluscan ancestor.

Wingstrand (1985) supports grouping the sister groups Polyplacophora and Conchifera (the latter group including monoplacophorans) as a monophyletic unit, the "Testaria" (Salvini-Plawen, 1972; 1980; Lauterbach, 1983), itself a sister group to the either mono- or biphyletic aplacophoran mollusks (i.e. the Caudovoveata and the Solenogastres). In support of this view, Wingstrand describes many testarian synapomorphic features including the radula and radular apparatus, the velum, the subradular organ, the large pharyngeal diverticula, the large digestive gland, the coiled intestine, the eight pairs of pedal retractor groups, and the already mentioned similarities of the heart complex. As Wingstrand (1985) has noted, even if as he has concluded, metamerism is primitive for testarians, it is difficult to determine whether a basic metameric organization is a plesiomorphic condition for testarians, present also in a protostomian ancestor or, alternatively, if metamerism is a synapomorphy for testarians. Only the "Aplacophora" are available for outgroup comparison and their nonmetameric condition could either be a primitive molluscan feature or attributed to convergent evolution due to a vermiform habit or neotenic reductions resulting from small adult body size. The serial nature of all known molluscan radulae might provide insight on the issue of metamerism but, not surprisingly, there is little general agreement on the features that are primitive to a radula. First, there are obviously two issues concerning the presumed serial or nonserial nature of the primitive radula, differing in whether the "metamerism" is bilateral (left and right) or longitudinal (serially repeated rows). Nierstrasz (1905) and Boettger (1955, 1959) proposed that the basic ancestral radula was bipartite (i.e. in two parts, symmetrical left and right) and distichous (i.e. arranged with two matched teeth in each longitudinally repeated row), while Salvini-Plawen (1972, 1978, 1981, 1985) contended it had a broad monostichous form. Meanwhile, Minichev and Sirenko (1974) and Ivanov & Tzetlin (1981) attributed to the Aplacophora and Polyplacophora a primarily monstichous radula and to the Conchifera a polystichous (Minichev and Sirenko, 1974) or a distichous (Ivanov & Tzetlin, 1981) radula.

Because aplacophorans have been regarded as the one or two earliest diverging of extant molluscan lineage(s) (i.e. Wingstrand,

1985) and because they have the simplest adult radula, the aplacophoran radula might be especially appropriate to consider. However, the highly specialized and diverse modes of feeding of many aplacophorans, especially those that are interstitial, could confound this conclusion. For example, about 25% of known members of the Solenogastres (Neomeniomorpha) lack a radula, using enzymatic secretions of a protrusible foregut to dissolve cnidarian tissue (Salvini-Plawen, 1985). The Caudofoveata (Chaetodermomorpha or Chaetodermatida) include several genera with a distichous radula and several that display a specialized feeding apparatus of disputed construction, whereas the distichous tooth pattern prevails unequivocally in members of the Solenogastres that have a radula (Salvini-Plawen, 1978). Moreover the radula of both aplacophoran groups clearly shows features of a bipartite construction: The teeth attach to a radular membrane which is often split medially, perforated by a series of slits, or is fused together from two ribbons (Heath, 1905; Salvini-Plawen, 1978; Scheltema, 1981; pers. comm. 1984; contrast with Hyman, 1967).

We have presented evidence that from the start the juvenile chiton radula is in most cases polystichous, not monostichous as believed by Minichev and Sirenko (1974). However, we believe the issue is much more fundamental than this distinction. Even if it could be shown that certain mollusks (i.e. two species of Solenogastres as claimed by Salvini-Plawen, 1985) pass through a monostichous stage in their radular formation, this would not necessarily indicate the primitive radular condition of a presumed early molluscan ancestor. Comparative ontogenetic studies might reveal the initial ancestral state (i.e. "Von Baer's laws") but this assumes that early ontogenetic stages are less prone to modification than later stages or, stated differently, the initial expression of a morphological trait reflects more accurately than later expressions an ancestral condition (Kluge and Strauss, 1985). In practice, testing this assumption requires outgroup comparison (Kluge, 1985) which in the present case is difficult because it would require comparisons of radular ontogeny with the ontogeny of a structure presumed to be homologous to the molluscan radula in a non-molluscan outgroup. Moreover, it would be mistaken to assume that the initial state of a juvenile chiton radula must correspond to the adult radula of a hypothetical ancestral mollusk. Instead, the juvenile condition of chitons is better compared to the ancestral juvenile condition, and simplicity (i.e. few teeth or even a monostichous condition) attributed to the inherently small size of juveniles.

Thus, there is no longer any reason to postulate that the presumed ancestor of early mollusks was equipped with a monostichous radula as suggested by Salvini-Plawen (1985), Minichev and Sirenko (1974), and Ivanov and Tzetlin (1981). In addition to the uncertainties inherent in using early ontogenetic stages to infer a primitive condition, two facts are incompatible with the suggestion of an ancestral monostichous condition. First, the predominant radula type of the Aplacophora is distichous and basically bipartite, even if there is an initial connection in the juvenile radula as claimed by Salvini-Plawen (1985). Second, none of six genera of chitons hitherto examined (this paper and Minichev and Sirenko, 1974) reveal any sign of a monostichous stage in their radular development, except for the "monostichous" radula of the unidentified "trochophore" depicted in Minichev and Sirenko (1974). We would reinterpret this latter case as distichous, consisting of two longitudinal rows of incomplete L_2 teeth. Both the prevailing aplacophoran radula type and the ontogenetic sequence of the chiton radula lead us to propose a rather different basic feeding instrument in early mollusks. We conclude that it was bilateral or even bipartite with one or more pairs of longitudinal rows of teeth. It would be tempting to assume that such a radula represents the ancestral type for all mollusks, but this extrapolation needs to be tested with additional comparative studies.

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LA VARIABILIDAD DE *HEMICYCLA BIDENTALIS* (GASTROPODA, HELICIDAE)[1]

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RESUMEN

Se realiza un estudio biométrico de *Hemicycla bidentalis,* endémica de la isla de Tenerife (Archipiélago canario).

Es una especie extraordinariamente variable, tanto con respecto a la concha como al aparato reproductor, llegando a ser tan grandes las diferencias conquiológicas entre algunas poblaciones que éstas parecen pertenecer a especies diferentes. Sin embargo, al ser graduales estas variaciones y al no existir separación geográfica entre ellas, se concluye que el flujo genético no ha sido interrumpido. Solo existe una poblacion aislada geográficamente del resto en época reciente, pero no muestra indicios de un proceso de especiacíon.

La variabilidad de *H. bidentalis* está relacionada con los tipos de vegetación sobre los que vive (íntimamente relacionados a su vez con las correspondientes características climáticas y altitudinales), de los que los principales son la laurisilva, la zona de transición y la zona basal; esta variación se debe a la extraordinaria capacidad de adaptación de la especie al biotopo, al igual que occure con *Iberus gualtierianus* en la península ibérica, por lo que las poblaciones más diferenciadas son, simplemente, ecotipos de ella.

Key words: Helicidae; *Hemicycla;* variability; biometry.

INTRODUCIÓN

La variabilidad en los gasterópodos pulmonados constituye un fenómeno cuya existencia prácticamente era desconocida hasta el siglo 19, en el que ya comenzaron a publicarse algunos datos interesantes, como los de Kobelt (1881) sobre las poblaciones de *Murella* en Sicilia.

A lo largo de este siglo, en cambio, son bastante numerosos los trabajos sobre variabilidad. Boettger (1913) mostró una seriacion de conchas de diferentes taxones de *Iberus* de la Península Ibérica, entre el globoso *I. alonensis* y el aquillado *I. gualtierianus;* Pfeiffer (1931) y Rensch (1937) trataron mas de 30 variedades de *Murella,* indicando Rensch que las formas globosas y aquilladas podrían estar relacionadas con los climas secos y cálidos; Biggs (1959) concatena varias formas de *Eremina;* Heller (1979) también estudia en este sentido el género *Levantina;* Alonso & Ibañez (1978) muestran una seriación entre el aquillado *Iberus rositai,* que vive en una zona kárstica, y el globoso-subdeprimido *I. loxanus,* que se encuentra en los alrededores de esta zona; y Bartolomé (1982) revisa la literatura sobre este tema, añadiendo varios ejemplos de los géneros ya reseñados y algunos otros (*Tyrrheniberus, Rossmaessleria, Macularia,* etc.); López-Alcántara & cols. (1983, 1985) y Alonso & cols. (1985) realizan un estudio estadístico y biogeográfico de la variabilidad en el género *Iberus,* con *I. alonensis* e *I. gualtierianus,* concluyendo que ambas formas son ecotipos de la misma especie; y el caso más espectacular es el tratado por Woodruff (1978) y Woodruff & Gould (1980), que estudian la exuberante diversidad morfológica de las conchas de *Cerion* en las islas del Caribe y en Florida, indicando que el género está constituído por una serie de semiespecies variables y politípicas.

En Canarias, en la isla de Tenerife (Fig. 1), existe otro notable caso de variabilidad relacionada con el biotopo en *Hemicycla bidentalis* (Lamark, 1821) (syn. = *malleata* Férussac, 1821), que habita fundamentalmente en la zona montañosa de Anaga, al NE de la isla, en 3 tipos básicos de vegetación

[1]Notes on the Malacofauna of the Canary Island, Nr. 10; Nr. 9: Revision of the genus *Hemicycla* Swainson 1840 (Mollusca: Helicidae) from Tenerife: 1 n. subgen. and description of 3 new taxa. Bull. Mus. Paris (in press). Work supported by project 1692/82 of the "Comisión Asesora de Investigación Científica y Técnica" of Spain (CAICYT).

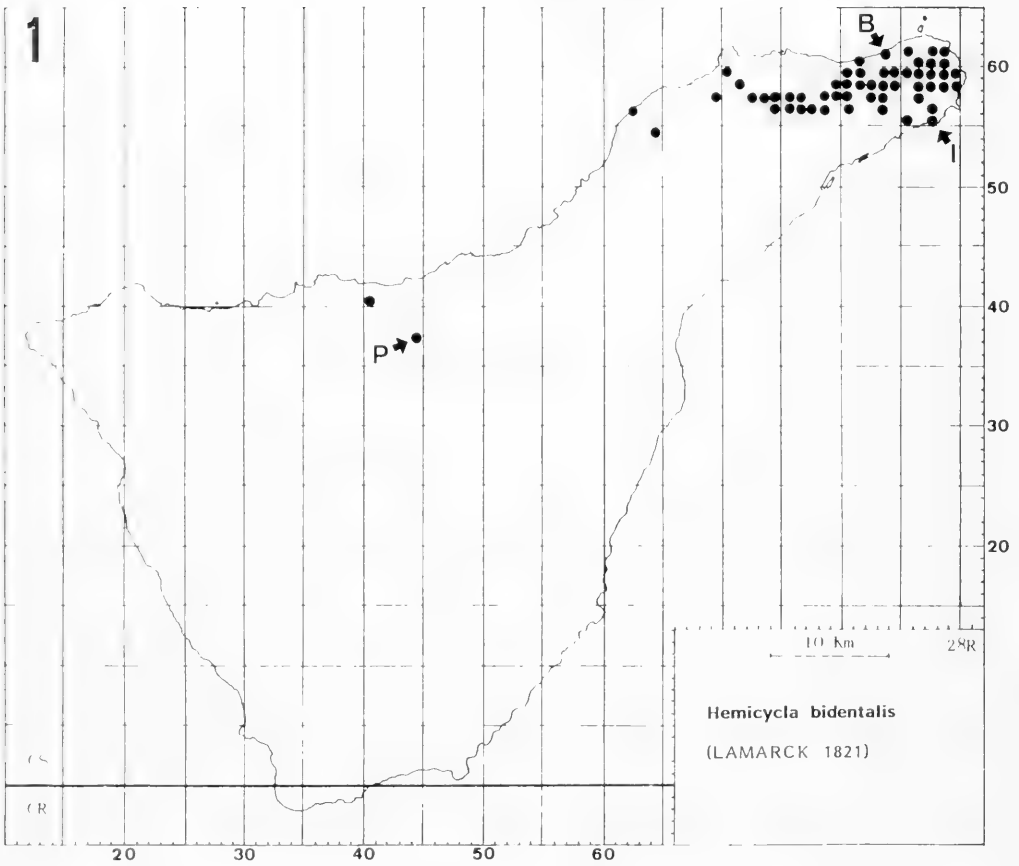

FIG. 1. *Hemicycla bidentalis.* Distribución geográfica. B: Benijo; I: Igueste de San Andrés; P: Palo Blanco; ●: localidades de procedencia del material estudiado.

(Fig. 2): el primero en las zonas altas, la laurisilva, bosque subtropical termófilo muy húmedo, relíctico del terciario; el segundo en las zonas bajas, el piso basal, formado por arbustos y matorrales xéricos de influencia africana, con muchas especies crasas del género *Euphorbia,* realizándose el paso de una a otra a través del tercero: el piso de transición.

Dentro de esta especie hay un conjunto amplio de poblaciones con características conquiológicas que a veces difieren de tal forma que a primera vista algunas de ellas parecen pertenecer a especies distintas (Fig. 3); esto ocurre al comparar la forma típica, de la laurisilva del macizo de Anaga, con la forma extrema del piso basal de Igueste de San Andrés y con un taxón fósil del Cuaternario parecido al de Igueste, *H. collarifera* Boettger, 1908, cuya localidad típica es Tejina, en la vertiente Norte del macizo de Anaga (Boettger, 1908). Pero entre ellas no hay aislamiento geográfico y, además, hemos observado un cambio gradual entre las 2 primeras a través de poblaciones intermedias, por lo que pensamos que no ha cesado el flujo genético y no pueden, por tanto, considerarse como especies distintas. Con menor espectacularidad, se diferencian también otras poblaciones, destacando la de Palo Blanco, que en la actualidad está aislada del macizo de Anaga por la acción humana (agrícola y urbanística), por una franja de unos 25 km de ancho (Fig. 1).

MATERIAL Y MÉTODOS

Para certificar su identificación, hemos comparado nuestro material (depositado en

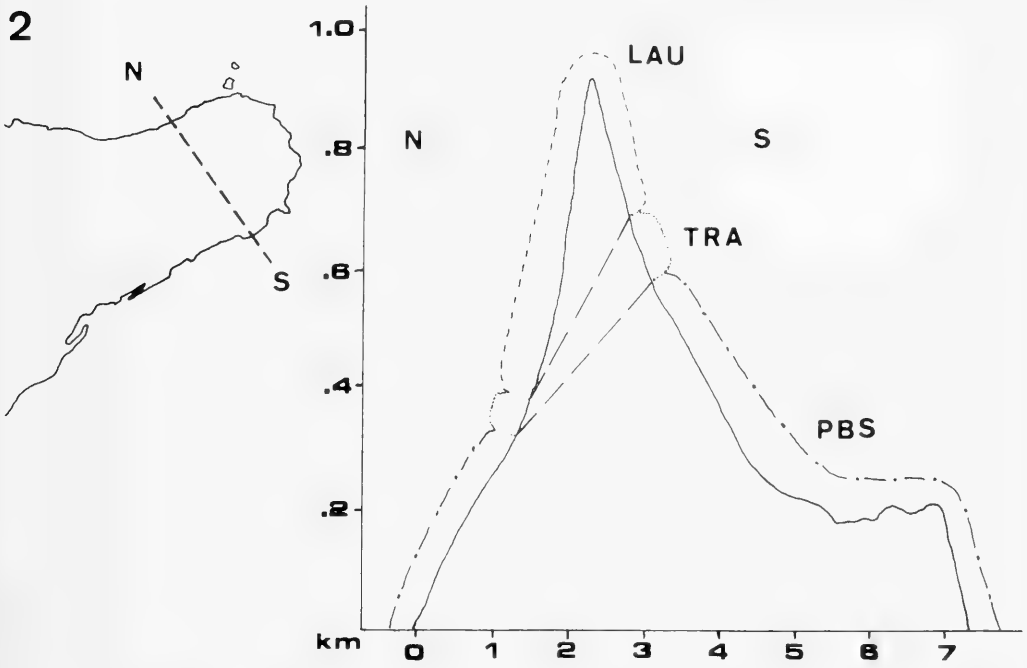

FIG. 2. Corte esquemático del macizo de Anaga, entre las localidades de Benijo e Igueste de San Andrés, mostrando la altitud a la que se encuentran los 3 tipos básicos de vegetación. LAU: laurisilva; TRA: piso de transición; PBS: piso basal; N: vertiente Norte; S: vertiente Sur.

el Departamento de Zoología de la Universidad de la Laguna, DZUL) con el de algunos museos: fotografías de los sintipos, del Muséum National d'Histoire Naturelle, Paris, enviadas por Mr. K. Groh (SMF); 1 concha de Santa Cruz, del Naturmuseum Senckenberg, Frankfurt/Main (SMF 33.635); 1 concha de La Paz (La Orotava; RNHL 50804) y 3 de Agua Garcia (RNHL 50805), del Rijkmuseum van Natuurlijke Historie, Leiden; y 22 conchas de Taganana, 10 del Barranco de San Antonio (La Orotava), 22 de las Mercedes y 20 de las cumbres de Anaga, del Museo Insular de Ciencias Naturales de Tenerife.

Para el estudio estadístico, realizado con el ordenador Digital VAX/VMS de la Universidad de la Laguna, se han recolectado 1698 ejemplares adultos (1550 conchas y 148 vivos), procedentes de diversas localidades (Fig. 1), de los que se extrajo el aparato reproductor en buen estado a 100 individuos.

Las variables analizadas fueron:

— De la concha: diámetro (D), altura (H), altura de la última vuelta (HU) y los índices D/H, D/HU y H/HU.

— Del aparato reproductor (longitudes): pene (PE), epifalo (E), flagelo (F), conducto común (CC), conducto de la bolsa copulatriz (BC), divertículo (DI) y los índices PE/E, F/PE, CC/PE, BC/PE, DI/PE, F/E, CC/E, BC/E, DI/E, F/CC, F/BC, F/DI, BC/CC, DI/CC y BC/DI (eligiendo siempre en el numerador la variable de media más alta para el conjunto de la población).

Se realizaron 5 análisis estadísticos:

1. Un análisis bivariante de la correlación entre todos los pares de variables posibles, en los casos en que se tenía información de todas ellas para cada ejemplar adulto (100 en total: Fig. 11), estudiándolos por separado según los 3 tipos básicos de vegetación en que se encontraron los poblaciones (LAU, laurisilva; TRA, piso de transición; y PBS, piso basal), obteniendo gráficos con las correspondientes nubes de puntos y los coeficientes de correlación producto-momento (r) entre ellas.

2. Otro análisis bivariante similar comparando sólo los datos conquiológicos de conchas adultas (1237 casos: Fig. 12), obteniendo además sus curvas de regresión respectivas, segun la expresión $y = ax^b$.

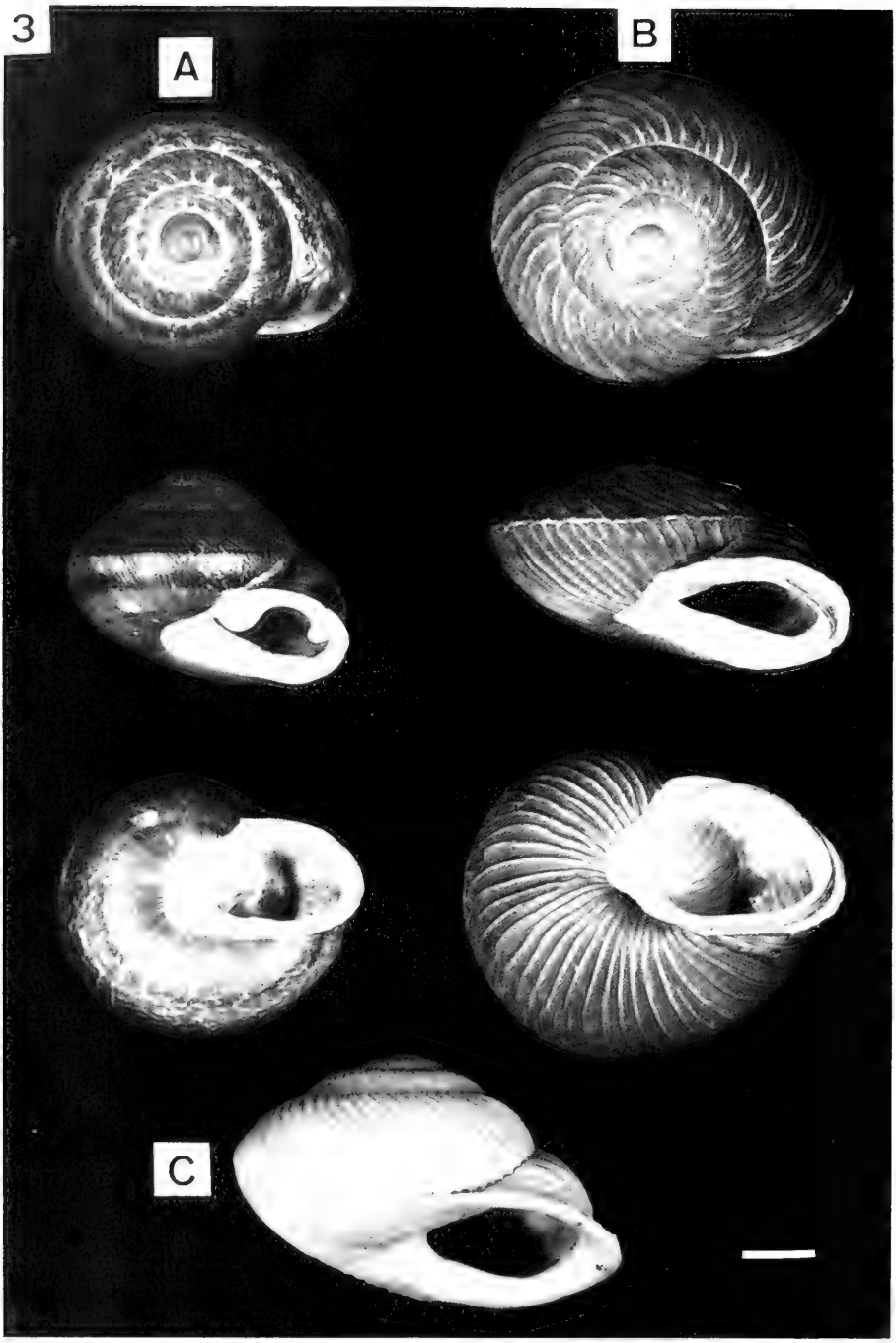

FIG. 3. *Hemicycla bidentalis.* A) Forma típica, de las cumbres de Anaga. B) Población de Igueste de San Andrés. C) *Hemicycla collarifera,* fósil de Bajamar (escala, 5 mm).

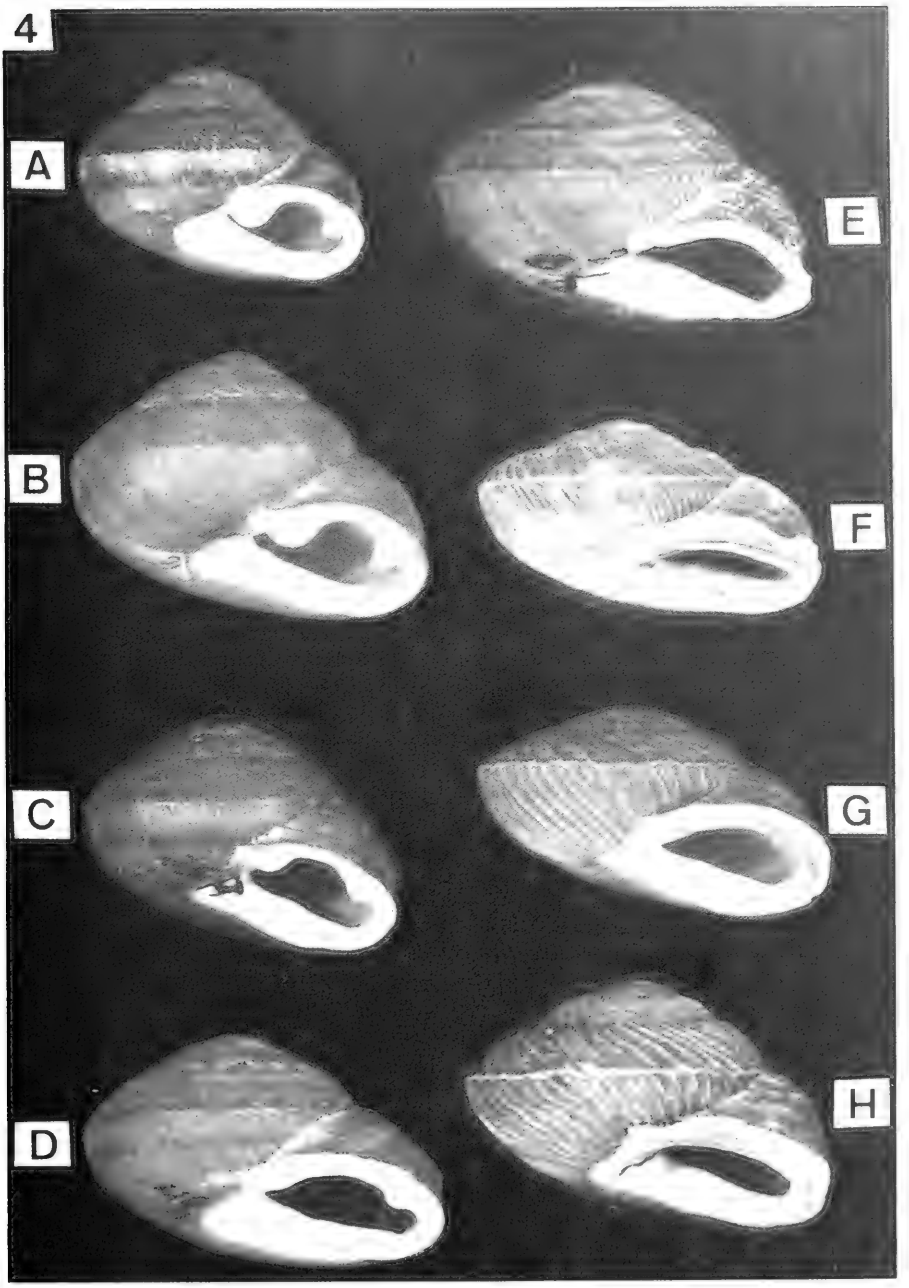

FIG. 4. *Hemicycla bidentalis*. Serie de conchas en las que se puede apreciar la transición desde la forma típica de la laurisilva de Anaga hasta la población extrema de Igueste de San Andrés. El cambio gradual se aprecia tanto en la escultura como en las denticulaciones (escala, 5 mm). A: Cumbres de Anaga (laurisilva, 800 m); B: Ijuana (laurisilva, 700 m); C: Bco. Roque Bermejo (piso de transición, 450 m); D: Benijo (piso basal, 200 m); E: Bco. de Anosma (piso basal, 200 m); F: Igueste de San Andrés (piso basal, 100 m); G: Igueste de San Andrés (piso basal, 80 m); H: Igueste de San Andrés (piso basal, 200 m). NOTA: Una serie similar a la fotografiada en esta lámina está depositada en las colecciones de la Academia de Ciencias Naturales de Philadelphia (ANSP 361423–361427).

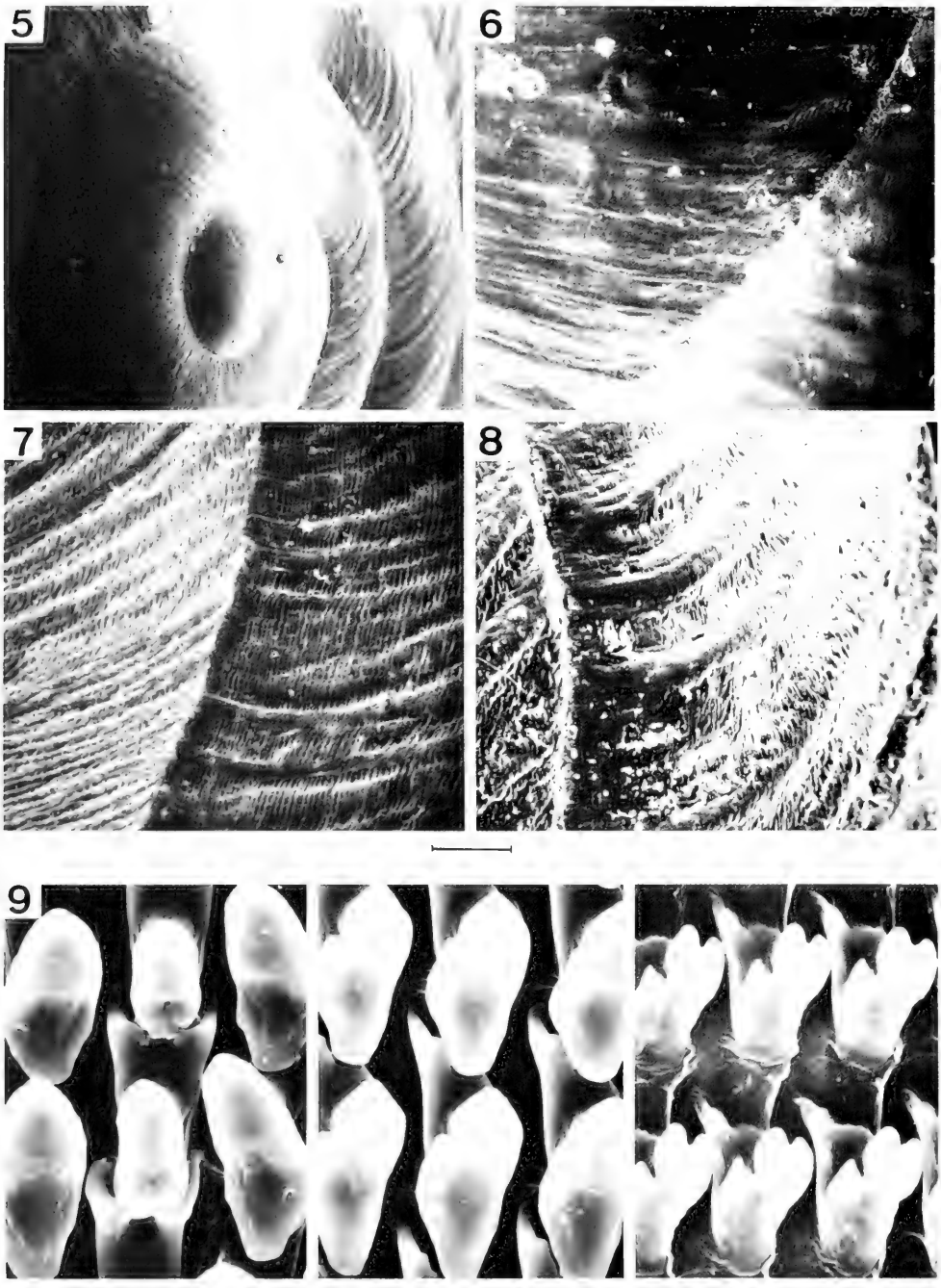

FIG. 5–9. *Hemicycla bidentalis*. 5) Detalle de la protoconcha de la forma típica. 6–8) Detalle de la penúltima y última vueltas de espira. 6) Población de las cumbres de Anaga. 7) Población de Benijo. 8) Población de Igueste de San Andrés. 9) Rádula; detalles de los dientes central, laterales y marginales. Escala: 5–8) 600 μm; 9) 25 μm.

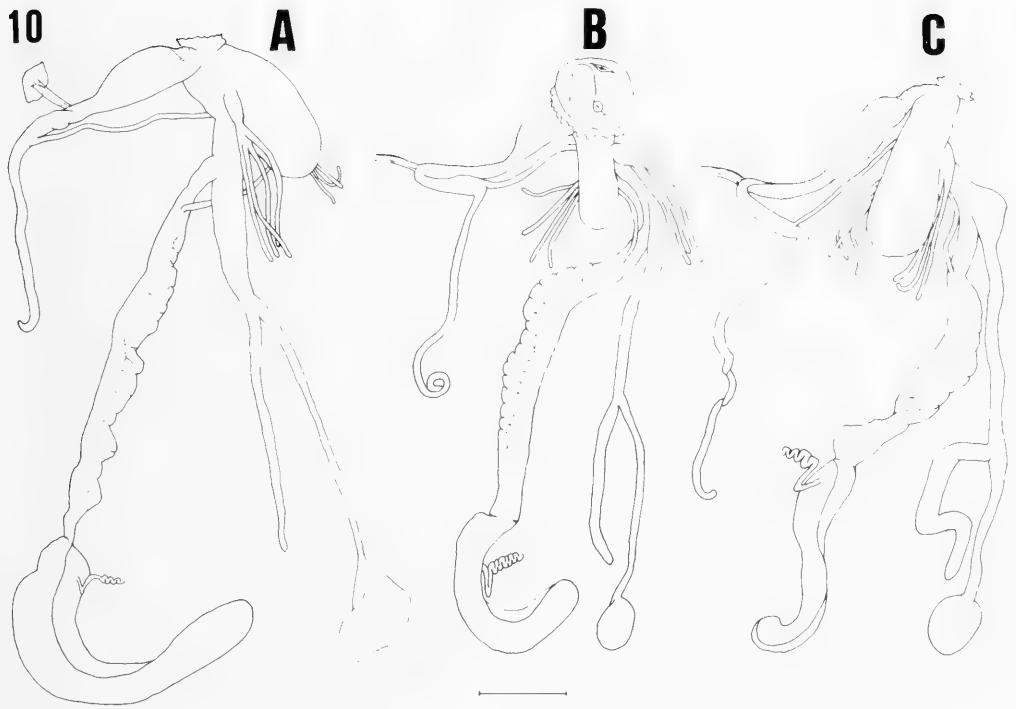

FIG. 10. *Hemicycle bidentalis.* Aparato reproductor (escala, 5 mm). A) Forma típica. B) Población de Igueste de San Andrés (el atrio y la porción anterior del pene están evaginados, mostrando la papila accessoria del pene). C) Población de Palo Blanco.

3. Un análisis discriminante por etapas (stepwise discriminant analysis, BMDP7M) entre las variables e índices conquiológicos (1237 casos) para los ejemplares procedentes de las poblaciones anteriores (LAU, TRA y PBS), pero dividiendo la primera en 2: LA1 (LAU de Anaga) y LA2 (LAU de Palo Blanco), para estudiar la posible segregación de esta última por su aislamiento geográfico actual con respecto a la primera. En estos mismos ejemplares se midió el grado de ornamentación (GO) en una escala arbitraria del 1 al 5, siendo mínimo (GO = 1) en conchas lisas y máximo (GO = 5) en las más rugosas y costuladas.

4. Otro análisis discriminante entre las variables e índices del aparato reproductor (100 casos) para comprobar si existen diferencias significativas entre las mismas poblaciones del análisis anterior.

5. Un test de t-student para igualdad de medias entre las variables e índices conquiológicos (incluyendo GO) y del aparato reproductor, para comprobar si existen diferencias entre LA1 y LA2.

RESULTADOS

A) Descripción de la forma típica y de sus modificaciones

1. Forma típica: Se encuentra en la laurisilva de Anaga y de Palo Blanco. La concha es gruesa, imperforada, globosa-cónica, con 4½ vueltas de espira, con suturas marcadas (Fig. 3A); su color es verdoso o amarillento claro en algunos ejemplares, pero sobre él se situan generalmente 5 bandas más oscuras, que a veces se fusionan entre sí, dándole un color oscuro uniforme; es ligeramente brillante, sobre todo en su superficie basal, que es más lisa, y en las 2 últimas vueltas tiene una maleación fina y uniforme (Fig. 6), mientras que las primeras poseen una débil costulación que se cruza con una leve estriación espiral existene en

TABLA 1. Matriz de correlación entre las variables. Los coeficientes mayores que 0.25 son significativos al nivel de P ≥ 99% y todos los son al nivel de P ≥ 95%, para n = 100. Concha; D: diámetro; H: altura; HU: altura de la última vuelta. Reproductor: PE: pene; E: epifalo; F: flagelo; CC: conducto común; BC: conducto de la bolsa copulatriz; DI: divertículo.

D	1								
H	.73	1							
HU	.85	.79	1						
PE	.38	.40	.46	1					
E	.23	.25	.23	.52	1				
F	.63	.47	.56	.54	.47	1			
CC	.61	.61	.61	.63	.53	.77	1		
BC	.40	.32	.38	.42	.21	.35	.83	1	
DI	.47	.40	.44	.45	.28	.56	.54	.54	1
	D	H	HU	PE	E	F	CC	BC	DI

TABLA 2. Dimensiones extremas y medias (en mm) de las variables estudiadas para el conjunto de las poblaciones. CV: coeficiente de variación; n: número de casos. Los demás símbolos utilizados son los mismos que en la Tabla 1.

	MAX	MIN	MEDIA	CV(%)	n
D	25.8	15.9	21.43	6	1237
H	17.9	11.1	14.45	8	1237
HU	13.2	8.5	10.84	7	1237
PE	13.5	4.5	9.50	16	100
E	8.0	2.0	3.91	25	100
F	24.0	8.5	17.17	18	100
CC	17.0	4.0	10.08	23	100
BC	16.0	6.5	11.42	15	100
DI	21.0	6.0	11.75	25	100

toda la concha, dando lugar en algunas zonas a la formación de gránulos. La protoconcha es más oscura y ligeramente rugosa (Fig. 5).

La última vuelta es muy globosa, ligeramente angulosa en su origen, pero sin quilla; presenta una estrangulación en las proximidades del peristoma, originando una pequeña gibosidad. El peristoma es blanco y presenta 2 fuertes calosidades, una en el punto de inserción del margen superior y la otra en el margen externo, estando el margen columelar engrosado por dentro.

2. Modificaciones hacia la costa: Al descender desde las cumbres de Anaga hacia la costa, se modifican la forma y la ornamentación de la concha: el color se hace más oscuro y uniforme y desaparece el brillo al aumentar la granulación y marcarse más la estriación espiral (Fig. 7); el tamaño también aumenta, al aumentar en ¼ el número de vueltas de espira, sin que aumente la altura; y las callosidades del peristoma se van haciendo más tenues (Fig. 4).

En los alrededores de Igueste de San Andrés (en el Sur de Anaga) se produce un cambio hacia una forma extrema, de aspecto completamente diferente (Fig. 3B): de forma gradual y en una distancia aproximada de ½ km (en la misma curva de nivel) desparecen las maleaciones, se mantiene muy fuerte la estriación espiral y se marca mucho más la costulación, originando fuertes costillas (Fig. 8); la forma se hace más deprimida y aquillada y la abertura es más redondeada, al desaparecer la callosidad del margen superior y reducirse a un vestigio la del margen externo (Fig. 3B). Esta forma se parece en su ornamentación y microescultura al taxón fósil *H. collarifera*, de la vertiente Norte de Anaga (Fig. 3C).

3. Anatomía interna: La rádula (Fig. 9) tiene en todas las poblaciones la estructura típica del género, con la siguiente fórmula: (C + 10–12L + 28–37M) × 110–130. El aparato reproductor (Fig. 10) varía extraordinariamente, tanto de unas poblaciones a otras como dentro de una población cualquiera.

B) Resultados de los análisis estadísticos

En la Tabla 1 se muestran los coeficientes de correlación entre todas las variables medidas; aun siendo significativas en su mayor parte, destaca el bajo valor de la mayoría de las correlaciones, sobre todo de las que presentan las variables del genital entre sí y con las demás. En la Tabla 2 se resumen las dimensiones extremas y medias de todas las variables para el conjunto de las poblaciones y los coeficientes de variación.

De los análisis bivariantes por poblaciones se deduce que no se pueden establecer zonas de discontinuidad entre ellas (Figs. 11 y 12), siendo destacable la enorme variabilidad de las diversas partes del aparato reproductor, tanto en conjunto como dentro de cada población.

Los análisis discriminantes muestran que las variables e índices conquiológicos que mejor caracterizan a cada subpoblación son D, H, y HU para la concha, y CC, BC/PE, F/E y F/DI para el reproductor, en este orden; las demás lo hacen con valores no significativos. Los coeficientes para las variables canónicas

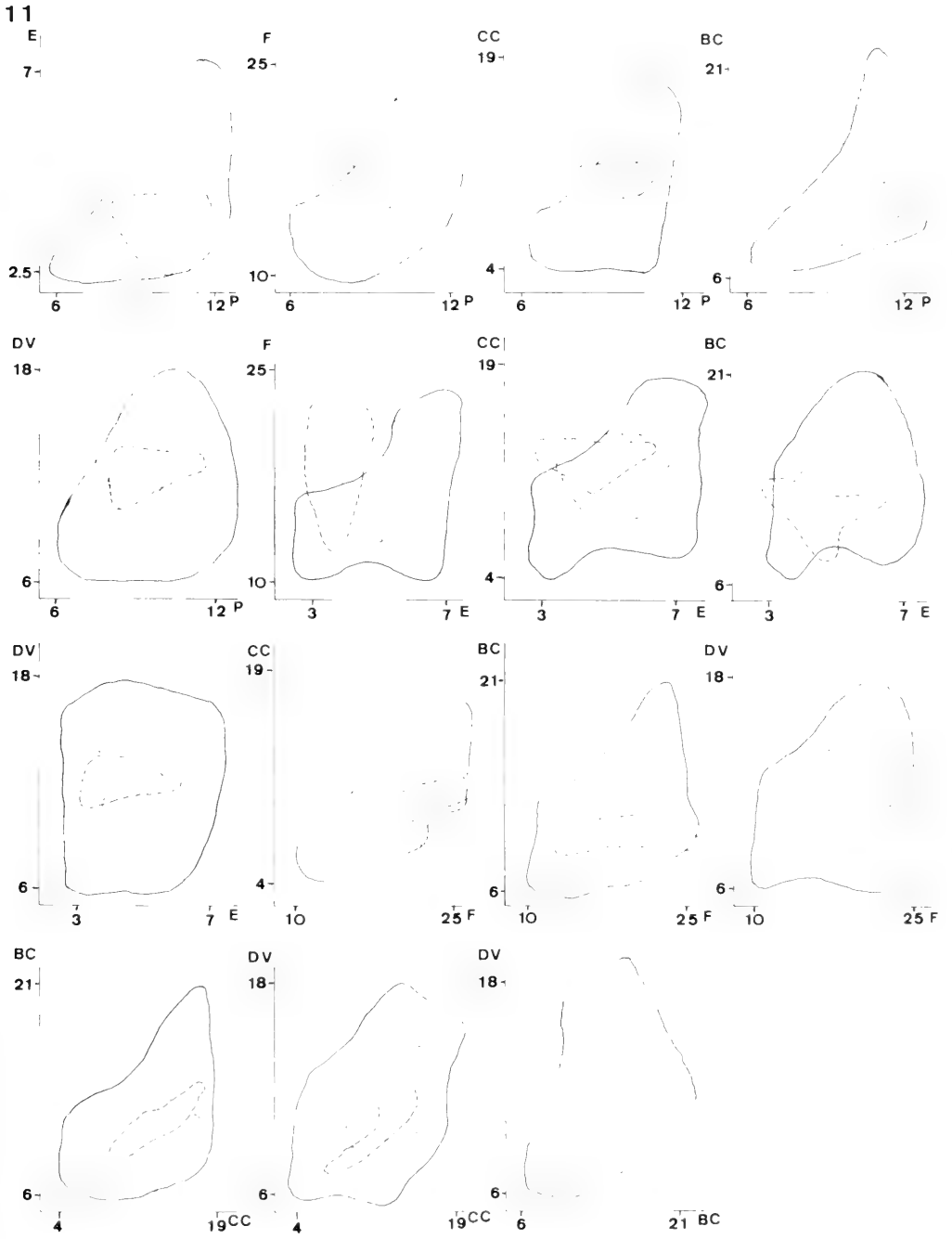

FIG. 11. *Hemicycla bidentalis.* Contornos de las nubes de puntos obtenidas al comparar las variables del reproductor 2 a 2. Línea de trazo continuo: laurisilva; línea de trazo discontinuo: piso de transición; línea de puntos: piso basal (n = 100 casos).

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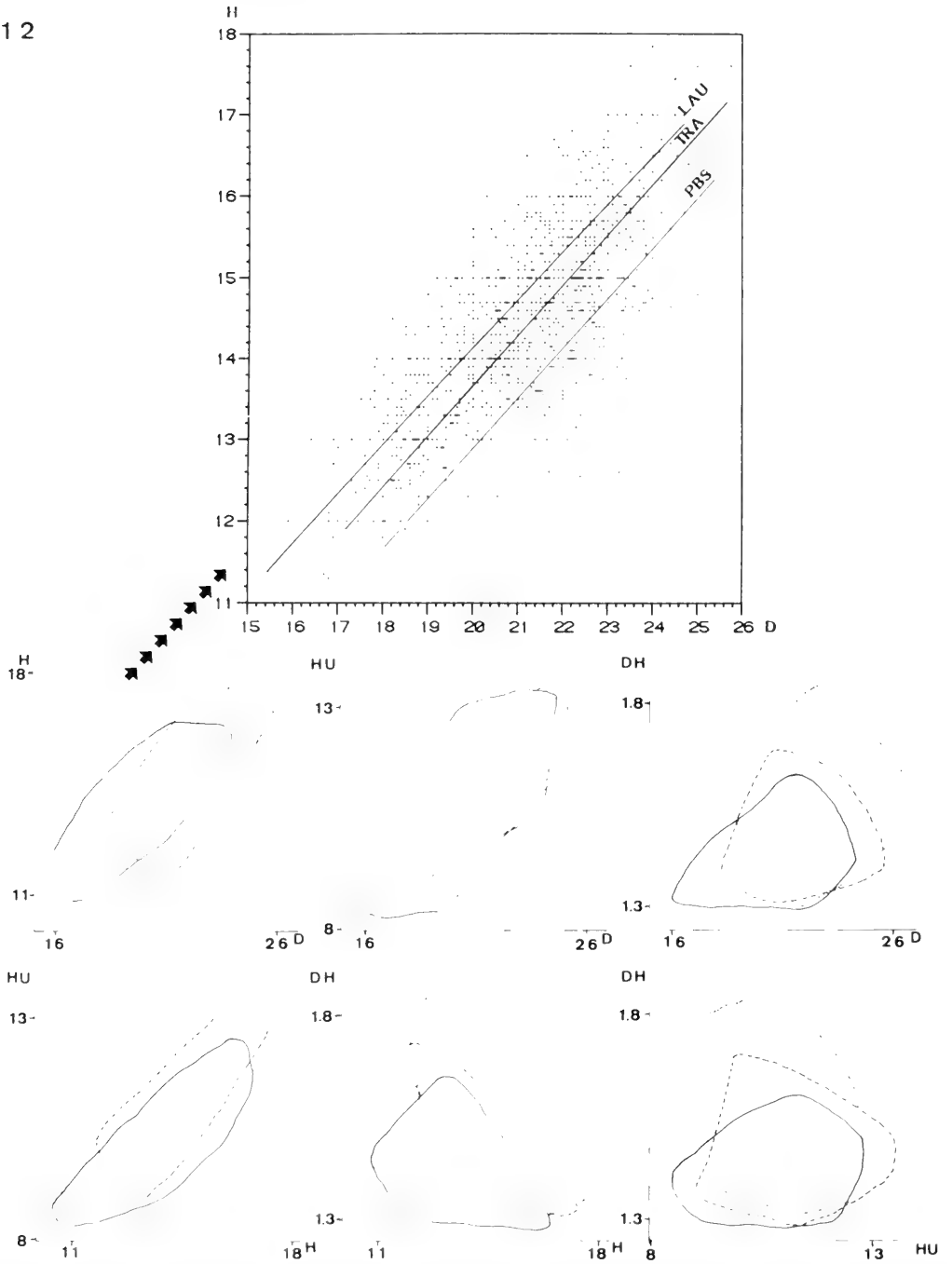

FIG. 12. *Hemicycla bidentalis*. Contornos de las nubes de puntos y líneas de regresión obtenidas para las variables conquiológicas. Los símbolos son los mismos que en la Fig. 11; DH: índice D/H (n = 1237 casos).

TABLA 3. Coeficientes de los análisis discriminantes de la concha y del aparato reproductor para las variables canónicas I, II y III; las demás variables no se indican, al no ser suficientemente discriminantes. PA: porcentaje de la dispersión total explicado por cada variable canónica. Los demás símbolos utilizados son los mismos que en las Tablas 1 y 2.

	CONCHA (n = 1237)				REPRODUCTOR (n = 100)		
	I	II	III		I	II	III
PA	88.8	10.9	0.3		67.9	28.0	4.1
D	1.12	0.24	0.17	CC	–0.40	0.13	0.14
H	–0.64	–0.18	1.52	BC/PE	0.18	2.73	4.95
HU	–0.62	–1.42	–2.02	F/E	0.30	0.71	–0.57
				F/DI	–1.51	0.56	0.31

TABLA 4. Porcentajes de clasificación correcta obtenidos en los análisis discriminantes para las variables canónicas de la tabla 3. LA1: laurisilva de Anaga; LA2: laurisilva de Palo Blanco; TRA: piso de transición; PBS: piso basal; PCOR: porcentaje correcto; n: número de casos.

	CONCHA						REPRODUCTOR					
	PCOR	LA1	LA2	TRA	PBS	TOTAL	PCOR	LA1	LA2	TRA	PBS	TOTAL
n		318	202	316	401	1237		14	48	12	26	100
TOTAL	61.5	25.7	16.3	25.6	32.4	100	69.0	14.0	48.0	12.0	26.0	100
LA1	66.3	66.3	16.3	14.1	3.3	100	78.8	78.8	7.8	3.8	9.6	100
LA2	64.7	12.9	64.7	20.0	2.4	100	85.7	9.5	85.7	0.0	4.8	100
TRA	46.1	13.5	18.0	46.1	22.4	100	38.5	23.1	15.3	38.5	23.1	100
PBS	67.7	4.3	6.4	21.6	67.7	100	35.7	14.3	14.3	35.7	35.7	100

I, II y III se exponen en la Tabla 3 y los porcentajes de clasificación correcta según las funciones de clasificación se exponen en la Tabla 4. Se observa que para la concha el 61.5% de los casos se clasifican en su grupo correspondiente, existiendo por tanto algo mas de una tercera parte que puede ser clasificada en un grupo diferente al suyo; para el reproductor, en cambio, lo hace el 69%, por lo que las variables del reproductor son ligeramente más discriminantes que las conquiológicas. En la Fig. 13 se representa el contorno de las nubes de puntos en el plano definido por las 2 primeras variables canónicas, en donde está representado el 99.7% y el 95.9%—respectivamente—de la dispersión total, y los centroides de cada grupo para la concha (Fig. 13A) y para el aparato reproductor (Fig. 13B). En ambos casos las 4 poblaciones se solapan entre sí, correspondiéndose los solapamientos entre cada nube de puntos y las demás con los porcentajes de la Tabla 4. Prácticamente no existe ninguna frontera o separación entre los 4 grupos, aun empleando las 2 combinaciones lineales (variables canónicas) de las variables que más separan los 4 grupos entre sí; para ambas figuras, el centroide de LA2 se separa de los demás tanto como el de LA1; también es destacable la proximidad de los centroides de TRA y PBS en el caso del reproductor (Fig. 13B).

En la Tabla 5 se muestra la variación del grado de ornamentación según el tipo de vegetación, cuyo valor aumenta desde la laurisilva hacia la costa; esta variable, junto con el desarrollo de las callosidades del peristoma, son las más conspicuas en la diferenciación de las poblaciones, aunque son difíciles de cuantificar para su estudio estadístico; también se indican las medias por poblaciones y el resultado del test de t-student para comparar las 2 poblaciones de laurisilva (LA1 y LA2), que muestra que las diferencias entre ambas poblaciones son significativas salvo para las variables BC y DI, siendo mayores los valores correspondientes a LA2.

DISCUSIÓN

Tras el examen de los coeficientes de correlación mostrados en la Tabla 1, se observa que éstos son bastante bajos

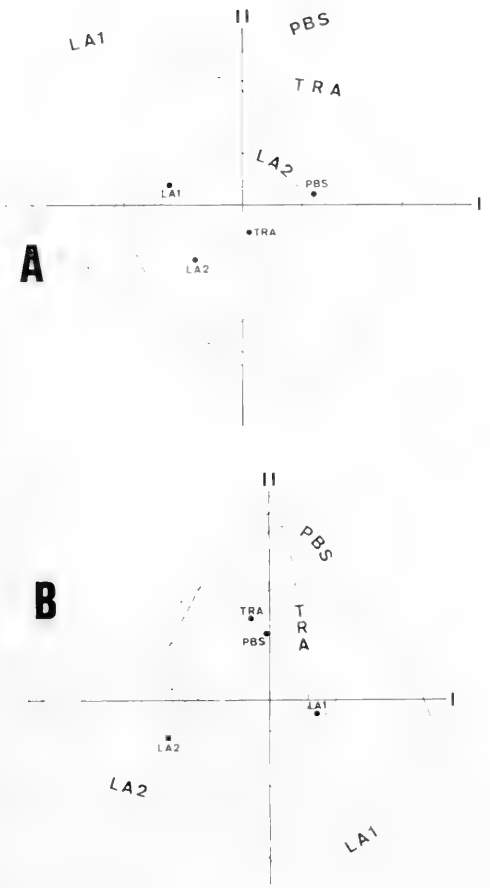

FIG. 13. *Hemicycla bidentalis*. Resultados de los análisis discriminantes para la concha (A; n = 1237) y para el aparato reproductor (B; n = 100); se muestra el contorno de las nubes de puntos en el plano definido por las 2 primeras variables canónicas y los centroides de cada grupo. LA1: laurisilva de Anaga (trazo continuo); LA2: laurisilva de Palo Blanco (trazo de raya-punto-raya); TRA: piso de transición (trazo discontinuo); PBS: piso balsa (linea de puntos).

excepto entre CC-F y BC-CC y entre las medidas de la concha lo que indica que, salvo estas excepciones, la variabilidad de cada uno de los parámetros medidos no se relaciona con la variabilidad de los demás lo suficiente como para poder afirmar que son taxones diferentes. A esta misma conclusión se llega tras el exámen de las nubes de puntos obtenidas en los análisis bivariantes (Figs. 11 y 12), que poseen contornos generalmente redondeados sin que se

TABLA 5. Valores medios de las variables estudiadas, por poblaciones. •: diferencias significativas entre las medias de las dos poblaciones LA1 y LA2 al nivel de P ≥ 95%, segun el test de t-student para igualdad de medias. GO = grado de ornamentación; los demás símbolos utilizados son los mismos que en las Tablas 1 y 4.

	LA1	LA2	TRA	PBS
n	368	85	317	467
• D	19.89	21.30	21.92	22.34
• H	14.06	15.04	14.87	14.36
• HU	10.42	11.36	11.15	10.86
• GO	1.1	1.0	2.5	3.3
n	52	21	13	14
• PE	9.01	10.93	9.58	9.09
• E	3.49	5.36	3.38	3.80
• F	14.87	19.93	19.26	19.60
• CC	8.34	13.14	11.31	10.78
BC	11.15	11.19	11.69	12.48
DI	11.04	12.57	11.81	13.07

aprecie un alargamiento notable en ninguna dirección, observándose también un amplio solapamiento de las 3 poblaciones.

El mismo resultado se obtiene con los 2 análisis discriminates. El solapamiento de las 3 poblaciones de Anaga, junto con la inexistencia de aislamiento geográfico entre ellas, son evidencias de que no se ha interrumpido el correspondiente flujo genético. También se produce solapamiento con la poblacion LA2 de Palo Blanco, aislada recientemente.

Por lo que respecta a la concha (Tabla 3), se observa una tendencia al aumento de D desde las poblaciones de laurisilva a las de los pisos de transición y basal (Figs. 4 y 12); H y HU no experimentan, en cambio, una variación tan fuerte, presentándose la media más alta en las poblaciones del piso de transición.

En cuanto al aparato reproductor, se puede apreciar la enorme variación que experimentan sus conductos (Tabla 2), así como una tendencia al aumento en las poblaciones costeras con respecto a las de laurisilva de Anaga, tendencia que curiosamente está más acentuada en la población de laurisilva de Palo Blanco (Fig. 13B), de lo que podría deducirse que en esta última se está esbozando un proceso de especiación. Sin embargo, aunque las diferencias entre las medias de las 2 poblaciones de LAU son significativas

estadísticamente (como muestra el test de t-student; Tabla 5), son similares a las existentes entre las poblaciones de LAU, TRA y PBS de Anaga excepto en el grado de ornamentación, en que son mucho menores, por lo que las 4 coresponden sin duda a la misma especie; por otro lado, la variación del grado de ornamentación que exhibe *H. bidentalis* con respecto a los 3 tipos de vegetación puede interpretarse como una adaptación a cada biotopo.

Al no haberse interrumpido el flujo genético entre las poblaciones del macizo de Anaga, y al haberse diferenciado formas similares en el Cuaternario, todos estos cambios sólo pueden considerarse como el resultado de un proceso iterativo, iniciado a partir de una morfología común y debido a la extraordinaria capacidad de adaptación al medio ambiente de la especie, por lo que las poblaciones más diferenciades (como *H. collarifera* y la de Igueste de San Andrés) deben considerarse, simplemente, como ecotipos de *H. bidentalis.*

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ABSTRACT

THE VARIABILITY OF *HEMICYCLA BIDENTALIS* (GASTROPODA, HELICIDAE)

M. Ibáñez, J. Barquín, E. Cavero & M. R. Alonso

Hemicycla bidentalis is studied biometrically. It is a very variable species: the shell differences between the various populations on the Anaga massif are so great that at first sight some of them seem to be different species. However, the overlapping sets of data points from the different populations, and considering that they are not geographically separated, gives evidence that gene flow has not been interrupted. Only one population has become geographically isolated in recent times, but it shows no evidence of speciation.

The variability of this species is linked with the main types of vegetation among which it lives: the very wet laurisilva (evergreen laurel forest community), the transition zone and the arid lowland zone. It is due to its extraordinary adaptative capacity to the biotope, similar to the case of *Iberus gualtierianus* on the Iberian peninsula, that the most differentiated populations are in fact ecotypes of the same species

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ANATOMY AND HISTOLOGY OF THE ALIMENTARY TRACT OF THE SNAIL *THEBA PISANA* (GASTROPODA: PULMONATA)

Carmen Roldan[1] & Pedro Garcia-Corrales[2]

ABSTRACT

A morphological and histological description of the digestive tube of *Theba pisana* is given in this paper. Light-microscope observations demonstrated that the alimentary tract is divisible into six morphologically distinct regions: buccal mass, oesophagus, crop, stomach, intestine and rectum. The alimentary canal is lined by an epithelium of the columnar monostratified type, which shows three predominant cell types: unciliated, ciliated and glandular cells. Both the unciliated and ciliated cells possess a dense brush border of microvilli. The presence of glycogen and lipid droplets in their cytoplasm suggest they have an absorptive function. The ciliated cells are also related with the continued movement of food particles in the intestinal lumen. The mucous gland cells are likely responsible for lubrication of the luminal surface on the digestive tube and their secretions help to compact the faeces and cover the faecal pellets.

The major difference between the various regions of the alimentary canal is the relative number of the three epithelial-cell types. On the basis of our histological observations, there is evidence for the functional division of the *T. pisana* gut. The oesophagus appears to be specialized for movement of food particles. The crop serves as the storage organ. Notwithstanding, these regions of the digestive tube are most likely to be concerned with absorption. The stomach is very simple and lacks a gastric shield and style. The proximal ciliated and the secretory mid-intestine participate in digestion and absorption. The distal absorptive intestine and rectum are generally most important in faeces formation.

The alimentary tract is surrounded by a thin, continuous layer of loose connective tissue in the middle of which are few muscle fibres, obliquely and longitudinally arranged. This musculature is responsible for the peristalsis of the digestive tube.

Key words: anatomy; histology; alimentary tract; *Theba pisana.*

INTRODUCTION

Gastropods have been the subject of numerous studies. These studies have included gross anatomy observations and light microscopic investigations on their alimentary tracts. Yet surprisingly little is known about the histology. The digestive canal has been mainly studied in the prosobranch gastropods (Wu, 1965; Brown, 1969; Demian & Michelson, 1971; Martoja & Thiriot-Quiévreux, 1975; Sheridan *et al.*, 1978; Bolognani-Fantin *et al.*, 1982).

In the pulmonate gastropods studies on the gut are mainly concerned with the annexed glands, such as the digestive gland. Only scattered observations have been made on the histology of the alimentary tract in pulmonates. Studies on the anatomy and histology of the pulmonate digestive canal have been carried out by Carriker (1946a) in *Lymnaea stagnalis appressa* Say, Ghose (1963) in *Achatina fulica* Bowdich, Rigby (1963, 1965) in *Oxychilus cellarius* (Müller) and *Succinea putris* (Linné), Walker (1972) in *Agriolimax reticulatus* (Müller). Fragmentary results have been published on *Helix pomatia* Linné by Ferreri (1958a, 1958b, 1961) and Sumner (1965), and on *Arion ater* (Linné) by Bowen (1970). In addition, comparisons have been made of the gross anatomy of the alimentary tract of helicarionid, succineid and athoracophorid snails and slugs (Tillier, 1984).

To gain an appreciation of alimentary canal diversity in the gastropods, a stylomatophoran pulmonate, *Theba pisana* (Müller), was examined by light and scanning electron microscopy in this work. *T. pisana* is a herbivorous snail which crops bits of plants.

Preliminary observations on the anatomy and histology of the buccal mass of *T. pisana* have been carried out by Roldan & Diaz Cosin (1975). We have examined the structure of the epithelium in the digestive tube of *T. pisana.*

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The present study is aimed to reveal the regional differences in the alimentary canal of this species, as part of an ongoing study on its digestive system. Such a study is an important prelude to our attempt to correlate digestive activity with ultrastructural changes to the different cells of the digestive epithelium.

MATERIALS AND METHODS

Specimens of *T. pisana* were collected from Santander and Pontevedra (Spain). Individuals were transported to Department of Zoology, Complutense University, where they were maintained in a terrarium. The animals were fed lettuce.

To show the gross morphology of the digestive tract, the snails were anaesthetized in a 0.1% solution of chloral hydrate for 12 hr. The shell was then removed and whole animals were fixed in cold 10% neutral formalin for at least 24 hr. The snail was progressively dissected with the aid of a binocular microscope. When the digestive tract was uncovered, it was drawn under a camera lucida. Finally, the alimentary canal was opened longitudinally to observe its internal morphology. A reconstruction of the stomach was made from serial sections.

It was not possible to observe ciliary currents and food transport within the gut of live specimens.

Tissue preparation for light microscopy. The snails were directly immersed in the cold fixative fluids, and the alimentary tract was then rapidly removed from several animals. The fixation of small tissue samples, representing different regions of the digestive canal, were completed in the correspondent fixative fluid: Bouin's and Zenker media. 10% neutral buffered formalin and Baker's formol-calcium fluid. Fixed samples were washed, dehydrated in graded ethanols, cleared in xylene and finally embedded in paraffin (52° C). Serial sections, 3-7 μm thick, were cut on a Yung microtome, mounted on glass slides and stained with either hematoxylin-phloxin-light green, or with the methods of Heidenhain's azan and Mann-Dominici (Gabe, 1968).

In order to detect lipids, the tissue were fixed in cold Baker's formol-calcium fluid, then quickly dehydrated, embedded in paraffin, and sectioned at 6 μm. Staining was by Sudan black B (Gabe, 1968).

For evidencing glycogen, the tissue were fixed in cold absolute ethanol, and the sections were stained with periodic acid-Schiff's reagent (PAS) with maltase digestion as control (Gabe, 1968).

Tissue preparation for scanning electron microscopy (SEM). The alimentary tract was removed from several snails and divided into little segments, these were opened longitudinally to expose their luminal surface, and washed rapidly in three changes of cold sterile Locke's solution. Samples representing the different regions of the gut were then fixed immediately in 10% buffered (pH 7.4) formalin, dehydrated through a graded series of ethanol, transferred into acetone, and dried in a Polaron model E 3000 Series II critical point drying apparatus using liquid carbon dioxide. Dried samples were mounted on metal specimen stubs, then sputter coated with pure gold in a Polaron model E 5000 vacuum evaporator, and viewed with an ISI SX-25 scanning electron microscope operating at 25 KV.

RESULTS

The digestive system of *Theba pisana* consists of a buccal mass, two salivary glands, a slender oesophagus, the large thin-walled crop, a rounded stomach, the large digestive gland, a long twisted intestinal tract, with a proximal, mid and distal portion, the rectum, and the anus which opens on the right side of the body close to the pneumostome (Fig. 1).

The spheroid buccal mass is attached to the walls of the buccal cavity by numerous tensor muscles that insert onto its entire surface. This organ was previously studied by Roldan & Diaz Cosín (1975). The salivary glands originate from both sides of the dorso-posterior portion of the buccal mass, just above the pharyngo-oesophageal connection. These glands are relatively wide but elongate organs that extend back over the top of the oesophagus. They join the posterior end of the buccal mass by narrow ducts near the beginning of the oesophagus (Fig. 1). The digestive gland is the largest organ in the animal and comprises a substantial portion of the posterior region of the visceral mass. This gland surrounds the stomach and intestine, its two ducts opening into the stomach.

The alimentary canal is lined by a columnar monostratified epithelium which shows three predominant cell types at the light microscope level. It is composed mainly of columnar cells,

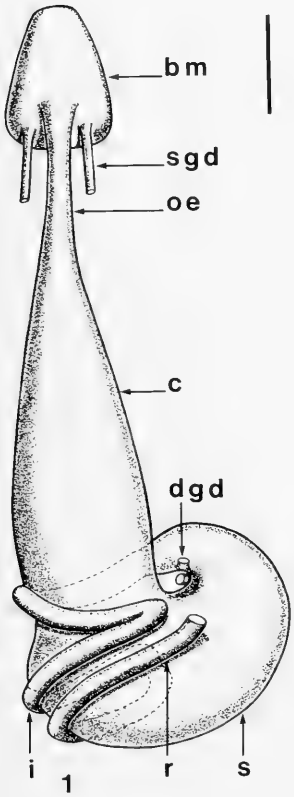

FIG. 1. *Theba pisana.* Dorsal view of digestive system. Salivary glands and digestive gland removed. bm, buccal mass; c, crop; dgd, duct of digestive gland; i, intestine; oe, oesophagus; r, rectum; s, stomach; sgd, duct of salivary gland. Scale bar 5 mm.

ciliated cells and gland cells (Fig. 2). The columnar and ciliated cells typically possess a brush border of microvilli. The whole digestive epithelium rests upon a thin, continuous layer of loose connective tissue interspersed with a few muscle fibres, which are obliquely and longitudinally arranged (Fig. 2).

Two major differences are observed between the various regions of the digestive tract. The first is the cell types present in the digestive epithelium, and the second the arrangement of their interior folds.

Oesophagus. The oesophagus (3.5–4.5 mm long) begins at the posterior upper aspect of the buccal mass, and merges with the crop (Fig. 1). The oesophagus forms no definitive, easily distinguishable pharyngo-oesophageal junction with the buccal mass. Rather, the oesophagus is formed by the

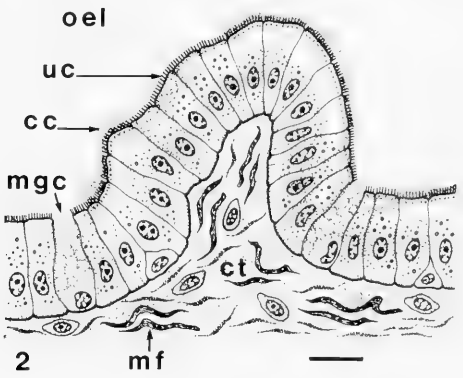

FIG. 2. Semi-schematic drawing of transverse section of oesophageal wall. cc, ciliated cell; ct, connective tissue; oel, oesophageal lumen; mf, muscle fibre; mgc, mucous gland cell; uc, unciliated cell. Scale bar 20 μm.

gradual tapering of the buccal cavity, and posteriorly it narrows to merge with the crop. The transition from the oesophagus to the crop is not abrupt (Fig. 1).

The oesophagus is round to oval in cross section, and its wall has internal longitudinal ridges (Fig. 3). These thick ridges are straight and extend into the anterior region of the crop.

The cell types seen in the oesophageal digestive epithelium consist of columnar, ciliated and glandular cells (Fig. 2).

The unciliated columnar cells are tall, narrow (20–30 μm high and 6–8 μm wide) and tightly packed (Fig. 4). These cells are abundant throughout the epithelium. Their apical regions have a prominent striated border about 2 μm thick, which consists of many, uniformly-distributed microvilli (Fig. 4) and stains positively by the PAS reaction. With the Heidenhain's azan method this brush border is stained blue. The cytoplasm of these cells is acidophilic and slightly granular in appearance (Figs. 2, 4). Their nuclei tend to occupy the mid to basal third of the cells, and they have an ovoid or spherical shape of about 5 μm in average diameter. These nuclei have numerous and disperse granules of chromatin, and one or two prominent nucleoli (Figs. 2, 4). These cells possess in their supranuclear cytoplasm abundant to lower amounts of large granules whose size varies from 0.3–1.5 μm. There are also lipid droplets as the Sudan black B stain reveals. When the sections are stained with the PAS technique

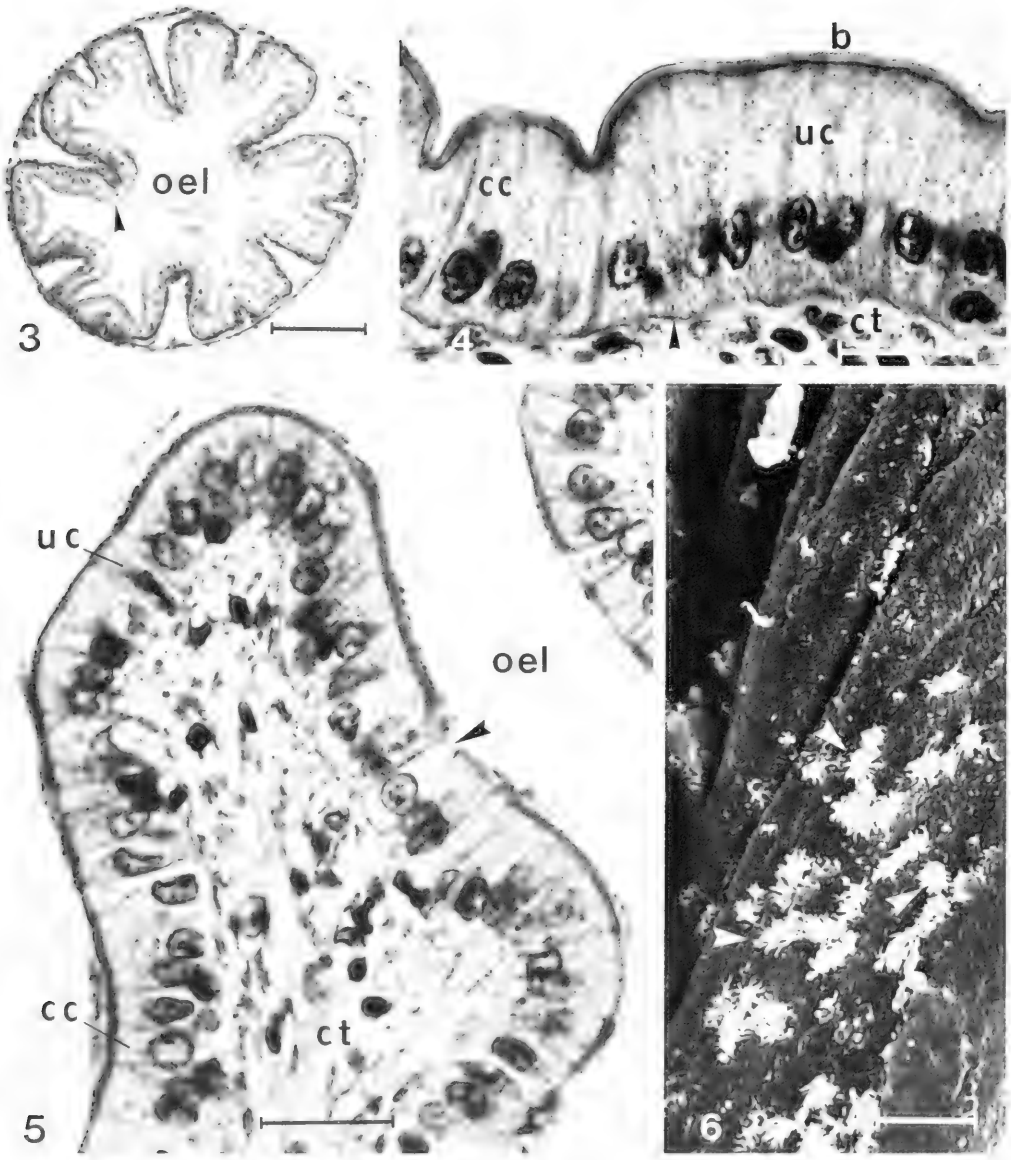

FIG. 3. Transverse section of the oesophagus. Note the prominent ridges (arrowhead). oel, oesophageal lumen. Scale bar 0.1 mm.

FIG. 4. Digestive epithelium of oesophagus, showing predominant unciliated cells (uc) and a clump of ciliated cells (cc). Beneath the epithelium is connective tissue (ct). The basement membrane (arrowhead) and prominent brush border (b) can be seen. Note the granular texture of cytoplasm. Scale bar 20 μm.

FIG. 5. Transverse section of ridge from oesophageal wall, showing abundant ciliated cells (cc), unciliated cells (uc) and mucous gland cells (arrowhead). Beneath the epithelium is well-vascularized connective tissue layer (ct). oel, oesophageal lumen. Scale bar 20 μm.

FIG. 6. Scanning electron micrograph of the luminal surface on an oesophageal ridge. Note the distribution of cilia in groups (arrowhead). Scale bar 0.1 mm.

with maltase digestion as control, the cytoplasm of these cells show the presence of glycogen which assumes different consistencies and location within the cytoplasm.

The columnar cells are intermingled with ciliated cells. The apices of these cells have brush border with cilia extending beyond the limit of the microvilli (Figs. 2, 5). The ciliated cells are tall and columnar with centrally-located, oval nuclei, and with cytoplasm very similar to that of the unciliated cells described above (Figs. 2, 5). Ciliated cells appear in groups where they protrude slightly into the oesophageal lumen, and the cilia present at the luminal surface of the oesophagus are found in groups instead of being evenly distributed throughout the surface (Fig. 6). In the anterior oesophagus, the ciliated cells are numerous, but over the folds they are more abundant. As the anterior oesophagus passes to the posterior one, the cilia decrease in number and they are found only on the tops of the folds.

The gland cells are goblet-shaped and lie between the remainder cells of the oesophagus digestive epithelium (Fig. 2). They are less numerous than the other cell types, and appear uniformly distributed throughout the epithelium. The nuclei in these cells are situated in the basal cytoplasm. They have an ovoid or spherical shape of about 3 μm in average diameter (Fig. 5). These densely reticulate nuclei possess no nucleoli, and stain more heavily than those of the other cell types. Their cytoplasm contains large numbers of granules, which are stained strongly by the PAS reaction. By Heidenhain's azan staining, the granule centre is light blue while the peripheral layer stains deep blue. These staining reactions of the granules denote their basophilic and acid mucopolysaccharide nature.

There is a thin layer of connective tissue surrounding the oesophagus. This layer contains many small muscle fibres (Fig. 2).

Crop. The oesophagus dilates and passes posteriorly to an inflated crop which is a sac-like enlargement of the digestive tract (Fig. 1). The transition from oesophagus to crop is gradual. This is a long (12 mm in length) wide tube, which is parallel to the longitudinal axis of the foot and enters the stomach. The posterior crop is not differentiated from the stomach, and it ends without any morphological discontinuity, except a slight annular constriction, in this organ (Fig. 1). The crop in comparison with the oesophagus has few longitudinal ridges, and these become less conspicuous so that the posterior half of the crop has no internal ridges.

The crop is internally lined with a non-ciliated epithelium. Two cell types constitute this epithelium: columnar and gland cells. The columnar cells are the most abundant throughout most of this epithelium. Both cell types are similar in morphology to those described in the oesophagus. But the cells of the crop digestive epithelium are taller than those of the oesophagus.

The crop is buried in a layer of loosely compacted connective tissue containing numerous scattered muscular fibres.

Stomach. The crop opens into the stomachal crop which continues to the stomach which is the most posterior region of the digestive tract, and is defined as the part of the alimentary canal which receives the two ducts of the digestive gland. The stomach extends farthest from the mouth, and forms a bend from which the intestine goes forward (Fig. 1).

The stomach is a rather globose to somewhat elongate curved organ; the most posterior part of this is the top of the stomachal pouch, whose posterior end is tapering to form a tube which becomes the intestine. The stomach lacks a gastric shield and a style. It possesses a large, non-cuticular smooth area in its internal anterior region lying adjacent to the crop opening (Fig. 7). In the stomachal pouch, near to its intestinal end, is a large area marked by many longitudinal ridges (Fig. 7).

The digestive gland surrounds the reflexed stomach and intestine. The two ducts of the digestive gland are almost circular in section. The anterior duct opens backward into the angle formed by the stomach and the proximal intestine, near to the entry of the latter (Figs. 1, 7). The posterior duct opens into the columellar side of the stomach. The duct openings of the digestive gland are round, and the gastric walls around them show radially arranged ridges (Fig. 7).

Two unequal typhlosoles extend from the duct openings of the digestive gland to the entry of the intestine (Fig. 8). The smallest one issue from the anterior duct, and the largest one from the posterior duct. The minor typhlosole ends at the beginning of the proximal intestine, and the major typhlosole continues along the proximal intestine (Fig. 7).

The epithelium that lines the stomachal crop is similar to that of the oesophageal crop,

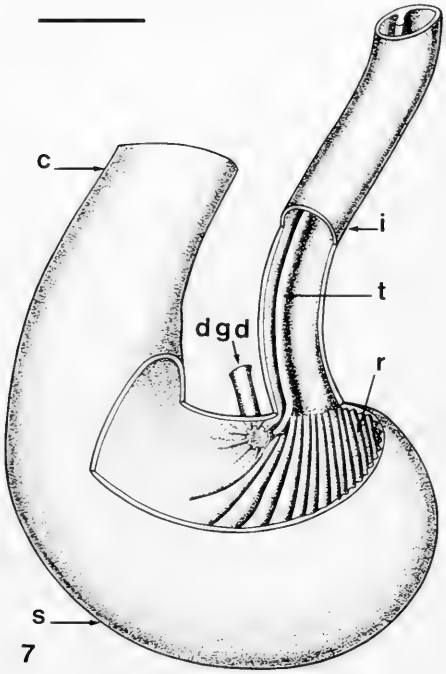

FIG. 7. Graphical reconstruction of the stomach and intestine. c, crop; dgd, duct of the digestive gland; i, intestine; s, stomach; r, ridge; t, typhlosole. Scale bar 2 mm.

but there are mucous gland cells present and the unciliated columnar cells are the most prevalent cell type. The cells lining the stomachal pouch are ciliated columnar (Fig. 9). The cilia on the ridges of this region are more abundant and larger than those elsewhere on the epithelium. Some mucous gland cells, similar to those described above, are intermingled with the ciliated cells (Fig. 9).

The connective tissue that surrounds the stomach is thicker and has more muscle fibres than that of the oesophagus and crop.

Intestine. The intestine opens from the columellar lower side of the stomach, curves to form a U, and passes under the oesophagus describing a half circle around it, to reach the left side of the body (Fig. 1).

The intestine goes backward over the dorsal surface of the stomach and coils before going to the ventral surface of the stomach where it reflexes again. The prerectal intestinal bend coils around the stomach and passes to its dorsal surface (Fig. 1).

For convenience the whole intestine is here divided into three regions, namely, the proximal, mid and distal intestine, since they differ histologically.

The major typhlosole, extending from the stomach, prolongs inside the proximal intestine (Fig. 7) where it ends gradually. This typhlosole has a median sheet of connective tissue with muscle fibres, and is tilted towards a side, delimiting a straight intestinal groove (Fig. 10). In addition to this, the proximal intestine has, at most, a few internal ridges which are slight (Fig. 10).

The digestive epithelium of the proximal intestine is strongly ciliated. The ciliated columnar cells are taller (30-40 μm in height) than those of the oesophagus, but morphologically similar to them. The cilia in this region are longer (4 μm in length) and more numerous than those of other regions of the alimentary canal (Fig. 11). Numerous gland cells of two types are intermingled with the ciliated cells. The first type is identical to the mucous gland cells of the oesophagus described above (Figs. 11, 12). The second type is similar in size and nuclear features, but possesses a cytoplasm different in some way. The greatest number of its secretory granules possess a slightly basophilic centre surrounded by a thin halo of more basophilic material. Intermixed with these granules are other strongly basophilic secretory granules which stain uniformly and intensely deep blue with the Heidenhain's azan method (Figs. 11, 12). The number of these homogeneous secretory granules varies from one to another gland cell of this type.

The digestive epithelium of the proximal intestine is invested by a thin layer of connective tissue containing a few muscle fibres.

The mid-intestine is distinguishable from the proximal intestine by the absence of internal ridges. Sections of the mid-intestine stained with the Heidenhain's azan method reveal its digestive epithelium comprised of ciliated and unciliated columnar cells, intense-staining, granular secretory cells, and lighter, highly vacuolated mucous gland cells (Fig. 13).

The unciliated and ciliated epithelium cells are identical to those described above. The latter decrease in number from the anterior to the posterior region of the mid-intestine, where they are found in clumps of two to three cells (Fig. 13).

The granular secretory cells constitute the most remarkable feature of the mid-intestine digestive epithelium. They have large, elongate (9 μm in average diameter) nuclei which

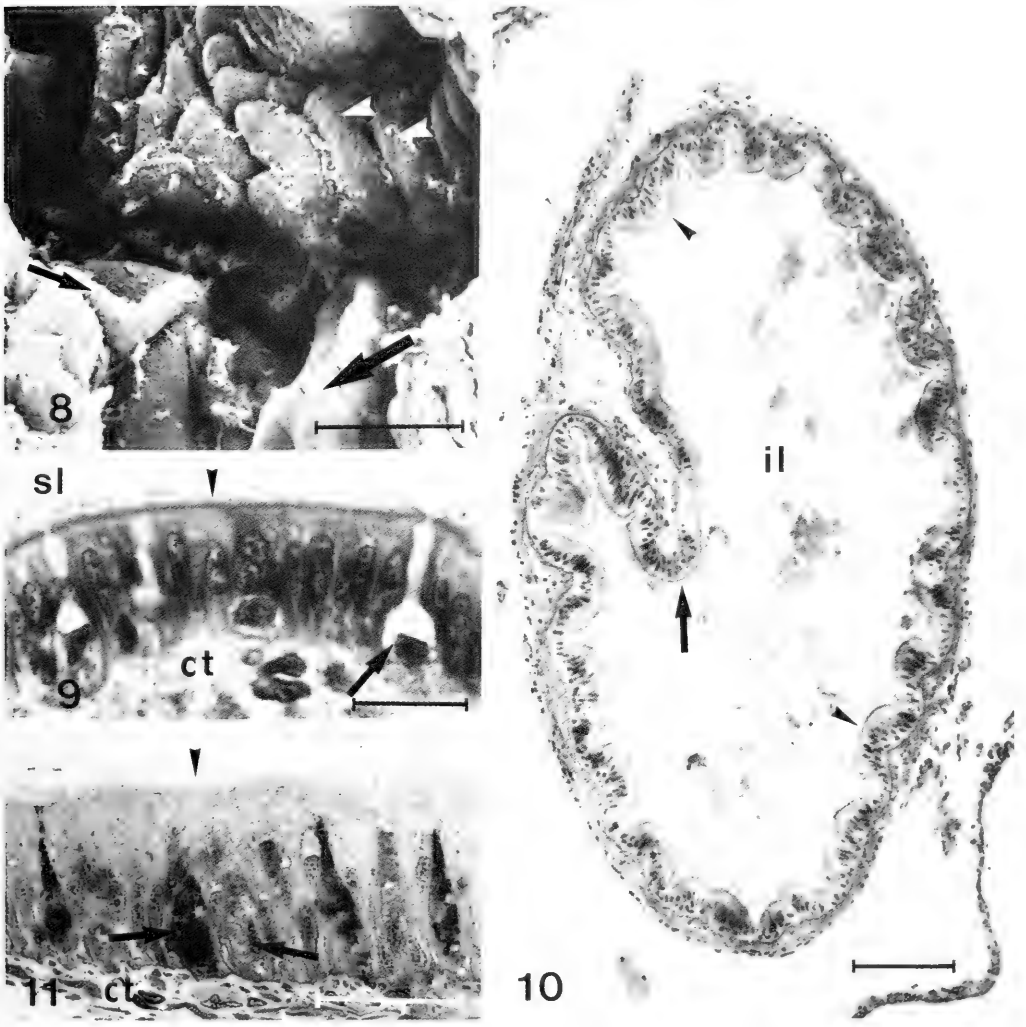

FIG. 8. Scanning electron micrograph of luminal surface on posterior region of stomach and entry of intestine. Note ridges (arrowhead), minor typhlosole (small arrow) and major typhlosole (large arrow). Scale bar 0.3 mm.
FIG. 9. Section of stomach posterior region. The epithelium displays columnar ciliated cells (arrowhead) and some mucous gland cells (arrow). ct, connective tissue; sl, stomachal lumen. Scale bar 20 μm.
FIG. 10. Transverse section of the anterior intestine demonstrating the titled typhlosole (arrow) and slight internal ridges (arrowhead). il., intestinal lumen. Scale bar 0.1 mm.
FIG. 11. Section of anterior intestine wall showing strongly ciliated epithelium (arrowhead) and mucous gland cells of two types (arrows). ct, connective tissue. Scale bar 30 μm.

are situated in the mid to basal third of the cells, and stain heavily. Numerous large granules which range in size and shape, fill almost totally the cytoplasm (Fig. 14). Two or more granules fuse to form larger ones. The secretory granules accumulate in the apical region of these cells, where they have a spherical shape and a maximum diameter of 1.5 μm. With the Heidenhain's azan technique, these granules show an orange-red homogeneous content; with the Mann-Dominici method, the same granules stain red-purple. The granules of these gland cells show a different aspect and dye affinities

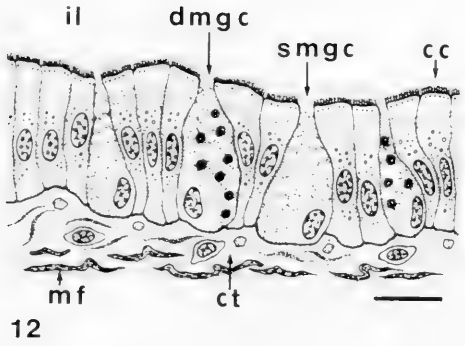

FIG. 12. Semi-schematic drawing of section of proximal intestine. cc, ciliated columnar cells; ct, connective tissue; dmgc, mucous gland cell with granules of two types; il, intestinal lumen; mf, muscle fibre; smgc, mucous gland cell with similar granules. Scale bar 10 μm.

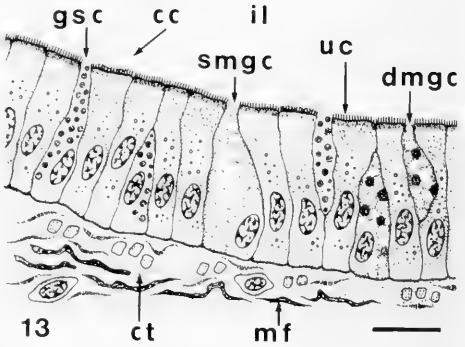

FIG. 13. Semi-schematic drawing of section of mid intestine. cc, ciliated columnar cells; ct, connective tissue; dmgc, mucous gland cell with granules of two types; gsc, granular secretory cell; il, intestinal lumen; mf, muscle fibre; smgc, mucous gland cell with similar granules; uc, unciliated absorptive cell. Scale bar 20 μm.

depending on their location in the cell. It is observed that granular gland cells empty their secretory granules into the intestinal lumen.

The mucous glandular cells in the mid intestine are similar to those of the proximal intestine (Figs. 12, 13).

The distal intestine is not differentiated from the mid- intestine, and have no internal longitudinal ridges. At the end of the distal intestine, the digestive epithelium and underlying connective tissue are elevated to form a single, longitudinal lateral fold, which is very patent (Fig. 15) and continues along the rectum.

The digestive epithelium of the distal intestine is mainly composed of unciliated columnar and gland cells, although isolated ciliated cells are also present. In contrast, the lateral ridge is densely covered by abundant ciliated cells. All these cells are similar to those described above.

The connective tissue surrounding the mid and distal intestine is similar to that of the proximal intestine, although more muscle fibres are present.

Rectum. The distal intestine continues to the rectum. The distal intestine and rectum go into the right side of the body.

The rectum is morphologically similar to the distal intestine; the transition from one region to another is gradual. The rectum is identical in diameter with the distal intestine, and neither show any internal morphological differences.

The inner surface of the rectum is smooth except for a longitudinal lateral ridge which rises in the distal intestine, prolongs inside the rectum and ends at the rectum posterior region.

The digestive epithelium of the rectum is comprised of ciliated columnar cells and mucous gland cells identical to those of the proximal intestine.

The rectum is surrounded by a thin layer of connective tissue having few muscle fibres. At the end of the rectum there is a sphincter around the anus.

Tiny, ovoid, faecal pellets found in the intestine and rectum are held in a fine mucous strand.

DISCUSSION

The *Theba pisana* digestive tube is composed of: a buccal mass, two salivary glands, an oesophagus, the crop, the stomach, the digestive gland, the intestine, the rectum and the anus. It is similar to that described by Rigby (1963; 1965) in *Oxychilus cellarius* and *Succinea putris*, and Walker (1972) in *Agriolimax reticulatus*.

A great deal of variation between animals in the size and number of folds within approximately similar regions of the alimentary canal was observed, but this may be correlated with how recently the animals had been feeding. We think that at least some of the folds may be temporary structures which disappear when the digestive tube wall is stretched, such as at times when the animal is taking in large quantities of food.

The digestive epithelium in *T. pisana* is

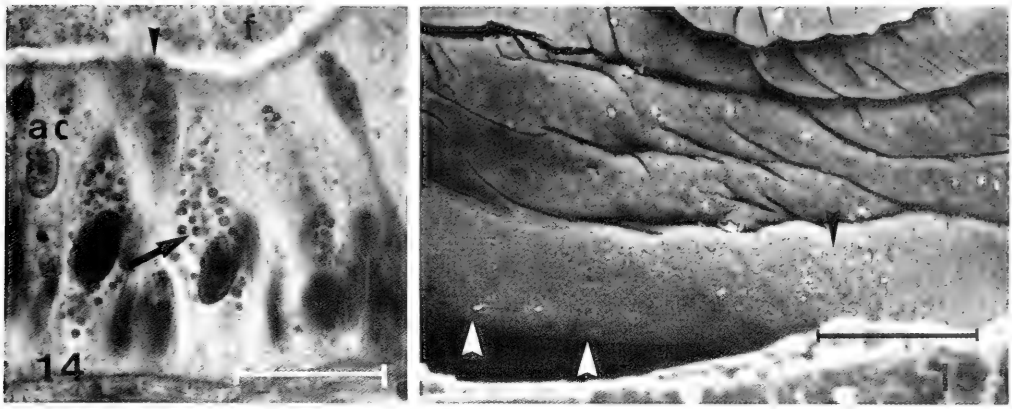

FIG. 14. Section of mid intestine wall showing granular secretory cells (arrows) and mucous gland cells (arrowhead). ac, columnar absorptive cell; f, food. Scale bar 20 μm.
FIG. 15. Scanning electron micrograph of luminal surface on distal intestine, demonstrating single, longitudinal lateral ridge (arrowhead). Scale bar 0.2 mm.

made up of columnar, ciliated and glandular cells.

Striated borders have been described as being related to the function of absorbing and transporting relatively large volumes of water, salts and protein over short time periods (Palay and Karlin, 1959). The dense brush border of the ciliated and unciliated columnar cells of the *T. pisana* digestive epithelium, in addition to the presence of glycogen and lipid droplets in their cytoplasm suggest they have an absorptive function. Carbohydrate and lipid absorption by the cells of the digestive epithelium has been demonstrated by Carriker (1946b) in *Lymnaea stagnalis* Linné, in which the oesophagus also present ciliated cells containing glycogen and lipids.

The presence of ciliated cells throughout almost the entire length of the *T. pisana* alimentary tract is likely a reflection of their assistance in the movement of food particles in the intestinal lumen.

The mucous gland cells of the *T. pisana* digestive epithelium produce large amounts of mucoid substances in the form of secretory granules. The highly positive reaction to PAS staining in light microscopic preparations is indicative of the carbohydrate nature of this material (Pearse, 1968). The fact that these granules, probably acid mucopolysaccharide in nature, form a continuous column from the base to the apex of cell suggests a continuous production of granules. Mucous gland cells are likely responsible for lubrication of the luminal surface on the digestive tube and may be important as stem cells for the digestive epithelium.

The composition of the mucous cell granules varies in the different regions of the *T. pisana* digestive tract. Thus in the oesophagus the texture of the granules is loose. In the intestine two types of mucous cells are observed, the first is similar to that of the oesophagus while the second type has granules with both loose and dense texture. It is conceivable that the texture of the mucous granules reflects differences in their glycoprotein composition. Alternatively it is possible that the differences in texture may represent granules in various stages of maturation. The present state of our preliminary ultrastructural investigations together with the limited number of histochemical tests does not allow us to decide between these alternatives.

Notwithstanding the different texture of mucous granules may represent granules in various stage of maturation, the present state of our preliminary ultrastructural investigations does not allow us to decide whether the difference in dye-binding capacity shown by the granules in the mucous gland cells may be referred to different stages in secretory activity, or rather to the existence of two different compounds.

The changes in the number of the different epithelial cell types are evident in each region of the *T. pisana* digestive tube. On the basis

of gross anatomy alone, there is evidence for the functional division of the alimentary tract in *T. pisana*.

T. pisana is a herbivorous pulmonate, whose oesophagus is devoid of a ciliated dorsal food groove, and of oesophageal glands. Our findings on the oesophagus anatomy in *T. pisana* agree with those described by Ghose (1963) for *Achatina fulica*.

The digestive epithelium of the *T. pisana* oesophagus possesses mucous gland cells with numerous granules, ciliated cells and columnar-absorptive cells. On the basis of our morphological study, gland cells with enzymatic secretions appear to be not present in the oesophagus of *T. pisana*.

The primary function of the oesophagus appears to be for the passage of food to the crop and this movement is no doubt aided by the release of mucous substances and the continued movement of food particles by cilia along the oesophageal epithelium. The abundance of ciliated cells in the *T. pisana* oesophageal epithelium may constitute a feature correlated with weak peristaltic action of the poorly-developed muscular coats of the oesophagus. The presence in such digestive epithelium of numerous cells with a well-developed brush border suggest that absorption may be other main function of the oesophagus.

T. pisana crop is devoid of chitinous plates or teeth; this fact suggests that it does not function as a gizzard. The digestive epithelium of *T. pisana* crop and stomachal crop is composed of gland cells and interspersed, unciliated supporting cells, indicating that muscle fibres of the subjacent connective tissue rather than cilia are probably responsible for directing food particles towards the strongly-ciliated sorting area in the stomach pouch.

The *T. pisana* crop, while serving as a storage and digestive organ, is also most likely to be concerned with absorption, as suggested by the presence of columnar-absorptive cells, the most abundant cell type throughout its epithelium.

T. pisana has neither gizzard nor gastric shield, and its stomach lacks a style. The efficient grinding structures of other snails could be substituted in *T. pisana* for the action of digestive gland enzymes, as it occurs in *Helix pomatia* in whose stomachal lumen Ferreri (1961) demonstrated proteolytic activity.

The absence of ciliated cells in the digestive epithelium of the stomachal crop in *T. pisana* is balanced by the well-developed muscular layer of its subjacent connective tissue.

The *T. pisana* stomachal pouch has a posterior grooved sorting area around the intestine entry and the digestive gland duct openings. The folds of this area could act in the selection of the food particle size. The two gastric typhlosoles in *T. pisana* are similar to those described by Ghose (1963) in *A. fulica*, Walker (1972) in *A. reticulatus* and Tillier (1984) in some helicarionid species. The function of cilia in the typhlosoles and sorting area of the *T. pisana* stomach appears to be to provide assistance in conveying food particles toward the intestine.

There are few data about the gastric functions in pulmonate molluscs. The exact roles played by the stomach in the digestive events are still not understood.

The *T. pisana* intestine is divided into the proximal ciliated, the mid secretory and the distal absorptive region. While the proximal and mid-intestine participate in digestion, the distal intestine is generally most important in absorption and faeces formation.

This study shows that the *T. pisana* proximal intestine possesses a digestive epithelium strongly ciliated and a musculature less-developed than that of the distal intestine which is devoid of ciliated cells. The proximal intestine typhlosole increases luminal surface and helps to move food particles.

The presence of granular secretory cells in the digestive epithelium of the *T. pisana* mid-intestine may be indicative of a higher secretory and therefore digestive activity in this region. Brown (1969) located enzymatic activity in the intestinal lumen of *Nassarius obsoletus* (Say). In the oesophageal and rectal epithelium of *Murex brandaris* (Linné) are present granular gland cells whose secretions are of enzymatic nature (Bolognani-Fantin *et al.*, 1982). These cells are morphologically similar to that described by us in *T. pisana* mid-intestine. This fact lends support to the proposal that this site is the another principal region for enzyme secretion, and both digestion and absorption of simple nutrients are carried out.

The digestive epithelium of the *T. pisana* distal intestine is entirely comprised of unciliated, columnar-absorptive cells and mucous gland cells. Absorption of different molecules by the intestine epithelial cells has been showed by Guardobassi and Ferreri (1953) and Sumner (1965) in *H. pomatia*, Rigby (1963) in *O. cellarius* and Brown (1969)

in *N. obsoletus.* On the basis of our findings and these data, it can be assumed that the primary function of the *T. pisana* distal intestine is one of absorption.

Formed faeces are of considerable importance for pulmonate gastropods because the anus is near the pneumostome, and firm faeces are less likely to foul this. Faeces formation generally involves the secretion of abundant mucus, the squeezing of the mucus and rejected material to form firm bodies and possibly some absorption of water.

A pellet compressor for the faeces formation has been described by Carriker (1964b) in *L. stagnalis appressa*, Demian & Michelson (1971) in *Marisa cornuarietis* (Linné), and Richards (1973) in *Biomphalaria glabrata* (Say). *T. pisana* produces solid faecal matter, but it lacks a definitive pellet compresor. Thus lubrication in the distal intestine and rectum would seem to be important, and an increased lubrication requirement would seem practical. The digestive epithelium of the *T. pisana* distal intestine and rectum contains many mucous gland cells which secrete mucus, helping to compact the faecal material. This abundance of PAS positive secretion in the intestine and rectum suggests that it provides the mucous covering of the faecal pellets.

The very ciliated, longitudinal fold of the *T. pisana* distal intestine and rectum is likely homologous to that of *Agriolimax reticulatus* (Walker, 1972) and *L. stagnalis* (Carriker, 1946b). Although there is undoubtedly some movement created by the peristalsis produced by the thin muscle layer, cilia would be particularly beneficial for the narrowed rectum extending from the distal intestine to the anus in *T. pisana.*

Not much is known of the details of rectal functions in gastropods. On the basis of the cell types predominant in the digestive epithelium of this region in *T. pisana* some food and water absorption appears to occur, but the main task is the condensation of the faeces which are well formed when released. The intestine and rectum in pulmonate snails have likely other functions, but more work is needed to define them.

ACKNOWLEDGEMENTS

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THE COMPARATIVE ECOLOGY OF FOUR SYMPATRIC LIMACID SLUG SPECIES IN NORTHERN IRELAND

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ABSTRACT

The distribution, feeding ecology, population structures and temperature sensitivity of the embryos of four sympatric species of *Limax* were examined. *L. pseudoflavus* and *L. flavus* were found mainly on walls, *L. maximus* on the ground and *L. marginatus* on trees. The feeding ecology was assessed both by direct observation and by faecal analysis. *L. maximus* showed a high proportion of vascular plant material in its diet whereas the other species fed predominantly on lichens. Examination of population structures indicate that *L. marginatus* is a univoltine, iteroparous species whilst the other three are polyvoltine and semelparous. At sites where either *L. pseudoflavus* or *L. marginatus* were found alone there was a significantly higher proportion of small individuals in the population than at a site where all four species occurred together. During incubation, *L. marginatus* embryos were the most tolerant of low temperatures whilst *L. maximus* and *L. flavus* were the most tolerant of high temperatures.

These results are discussed in the light of the behaviour and European distribution of the four species and it is concluded that there is substantial niche separation between *L. maximus* and the other species which is mainly based on its feeding preferences. The remaining species have a very similar feeding ecology but the substantial differences in life cycles and temperature sensitivity distinguishes *L. marginatus* from both *L. flavus* and *L. pseudoflavus.*

Key words: Gastropoda; ecology; feeding; niche separation; *Limax.*

INTRODUCTION

Species with close taxonomic affinities are usually also similar in their physiology and ecology. Where closely related species are sympatric it is to be expected either that adverse conditions act to keep coexisting populations below the carrying capacity in the areas of niche overlap or that there is a substantial element of niche separation (den Boer, 1986). In cases where the diets and distribution of closely related sympatric species have been examined niche separation has often been demonstrated (Pontin, 1982) and in closely related marine gastropods such factors as habitat, food size and activity patterns are involved (Spight, 1981). Where niche separation has been examined in terrestrial pulmonates however, such factors have been more difficult to identify (Cameron, 1978).

The genus *Limax* is represented in Ireland by five indigenous species. *L. cinereoniger* Wolf, 1803 is rare, but the others (*L. flavus* L., 1758, *L. maximus* L., 1758, *L. marginatus* Müller, 1774 and *L. pseudoflavus* Evans, 1978) are widespread and often numerous. These slugs are relatively large, have daytime resting sites to which they home (Gelperin, 1974; Cook, 1979, 1980) and in which they lay their eggs. They are distinguishable on external characteristics alone (Kerney & Cameron, 1979).

The general habitat types occupied by these *Limax* species have been described (e.g. Quick, 1960; Kerney & Cameron, 1979; Anderson, 1977; Evans, 1978). In summary, the habitats of *L. pseudoflavus*, *L. marginatus*, and *L. maximus* extend from woodland to gardens and walls. *L. flavus* on the other hand is more synanthropic and is rarely found away from buildings.

The most detailed information on pulmonate life cycles is available for slugs (Duncan, 1975), but this is largely for small pest species such as *Deroceras reticulatum* Müller (Hunter, 1968; South, 1982). The little information which exists on the life cycles of *Limax* spp. is largely anecdotal and this suggests that they mate and lay their eggs late in the year

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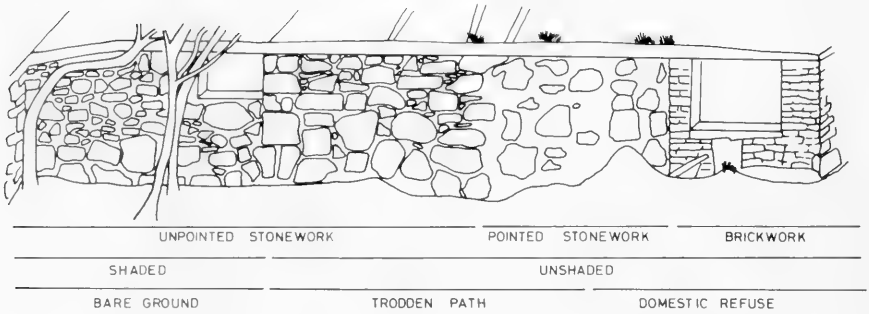

FIG. 1. Details of the main part of the Cranagh site. Small lengths of wall at both ends of this wall were included in the site. The main wall faces south-east.

(Quick, 1960). A more detailed study of *L. maximus* has shown that gonads undergo first male and then female maturation in response to increasing day lengths (Sokolove & McCrone, 1978) and therefore would be expected to lay cross-fertilised eggs in the autumn.

In Northern Ireland, slug communities often include three *Limax* species, and occasionally four. The objective of the present work was to compare aspects of the biology of these four species in an attempt to identify differences of ecological significance.

METHODS

General survey of habitats

Sixty-seven areas within the triangle whose corners are marked by Coleraine, Portrush and Portstewart (approximately 16 km^2) were inspected for *Limax* species in April and May, 1978. Surveys were undertaken at night when the animals were active and each area visited more than once. A quantitative study was not undertaken since activity is determined by the prevailing weather conditions (Ford, 1986) and only a small proportion of the areas could be adequately sampled on one night.

Frequency and size distribution of field populations

The mobility of slugs between and within sites was examined by collecting, freeze branding (Richter, 1976) and returning all the *L. pseudoflavus* (39) found on a small isolated group of stone walls. This site and neighbouring groups of stone walls were examined nightly for the following 22 nights until the brands became indistinct.

The populations of slugs at two field sites were monitored over the course of three years (1978-1980).

Both sites were isolated from other locations inhabited by *Limax* spp. Each site had well defined boundaries to facilitate the repeated sampling of the same area.

The Cranagh site (Irish grid ref. C841346) (Fig. 1) was part of a complex of stone farm buildings on the Coleraine campus of the University of Ulster. The study area comprised two exterior walls of an outhouse plus about 2 m of an adjoining garden wall. A strip of ground about 1 m wide in front of these walls and a small rubbish tip was included in the area. The tip was cleared in May 1979 and at the same time a wall of another stone building 10 m away was exposed. This wall supported a large population of *L. marginatus* and was also sampled after May 1979 on the same basis as the original site. It will be referred to as the uncovered wall.

The Kiltinny site was 1 km from the Cranagh site (Irish grid ref. C844354). It consisted of the shell of a small, derelict, stone building 3 × 3 m with walls 2 m in height, an adjacent wall 3 m long and 1.5 m high and a derelict field wall which was little more than a line of loose stones. The building had no roof and the site included both the outside and inside faces of the walls. The area sampled was roughly twice that of the original Cranagh site.

The walls of all three sites had a substantial covering of saxicolous lichen. Fresh higher plant material was always available at the base of the walls. Rotting vegetable matter was plentiful only at the Cranagh site.

Slugs were collected at night after emer-

gence from their day-time resting sites. For each sample the site was visited from dusk till dawn on three successive nights and all the slugs emerging on each night were removed. Each slug was stored individually in a plastic bag after its position and, if it was feeding, its feeding substrate had been noted. All slugs collected over the three day period were released on the fourth night at their points of collection. Estimates of the total population size were made using the rate at which the catches on successive nights declined (Zippin, 1956, 1958; Southwood, 1978). This method assumes that a constant proportion of the population is available for capture each night and that a large proportion of the total population is captured during the observation period.

For the first collection (May 1978) each slug was weighed in the field immediately after collection and then reweighed in the laboratory the following day. The two weights were not found to be significantly different (paired t-test $t = 0.62$, d.f. $= 120$, $p > 0.05$) and for subsequent collections all slugs were weighed in the laboratory except for a very few slugs found on the fourth night which were weighed in the field.

Nineteen samples were taken between May 1978 and September 1980. The observations on the life cycles of these species are derived from all these samples but those on the distribution within the site are only based on the 7 samples between May and November 1978. Such a restriction was necessary because the area around the site was unexpectedly cleared in May 1979.

Faecal pellet analysis

Faeces produced by slugs collected during the field sampling were stored in 70% alcohol. Faeces collected in November 1978, March 1979, May 1979 and June 1979 were analysed. Faecal pellets were suspended in water and sonicated until they broke up. Five aliquots of the resulting suspension were examined on a haemocytometer slide, the percentage cover of each food type estimated and the results averaged. Some pellets failed to break up on sonication and these were teased apart with fine needles before examination. Trial experiments with *L. pseudoflavus* fed on specific food items allowed the classification of faecal material into lichen, vascular plant material, *Pleurococcus* type algal cells, filamentous algal cells, fungal hyphae, and minerals. A very few pellets contained the remains of insects and earthworms but these were not frequent enough to be included in the classification.

Egg production in laboratory cultures

A culture of each species was set up in fibre glass bins $1 \times 1 \times 0.5$ m covered in clear polyethylene and containing a layer of soil and two plastic trays as homes. The bins were protected from extremes of temperature but open to ambient day lengths. Each bin was kept supplied with 'Readybrek' breakfast cereal and this diet was supplemented with leaf litter and fungi.

The cultures were established in May 1979 with 20 mature animals of each species collected in the field away from the main study area. In August 1979 the number of slugs in each culture was reduced to 10 to avoid symptoms of overcrowding. Whenever possible dead slugs were replaced immediately with individuals of similar size but this was difficult for *L. maximus* and *L. marginatus* during the winter months. The *L. maximus* culture was completely restarted twice (October 1979, July 1980) and the *L. marginatus* culture completely restarted once (July 1980).

Each bin was inspected almost daily and any eggs removed. In all species the eggs normally were laid in clutches in the homes. For the purposes of analysis a 'clutch' was considered to be a group of at least 5 eggs. Groups of less than five eggs were treated as belonging to clutches laid at about the same time. These small numbers of eggs were never added to clutches laid more than two days before or after their discovery. Occasionally two slugs laid at the same time in the same place, but unless two individuals were actually seen laying simultaneously all large groups of eggs were treated as one clutch.

Influence of temperature on egg development

The eggs were incubated in cooled incubators at four temperatures: 5, 10, 15 and 20° C. Clutches of more than 16 eggs were split into batches and each incubated at a different temperature. In practice this resulted in 28 batches from *L. pseudoflavus* containing 22.8 $\pm$ 1.9 (s.e.) eggs, 49 batches from *L. flavus* containing 17.7 $\pm$ 1.0 eggs, 23 batches from *L. maximus* containing 39.1 $\pm$ 4.6 eggs and 18 batches from *L. marginatus* containing

TABLE 1. The number of areas at which the various species were found.

Site type (N)	Number of areas containing				
	No *Limax*	*L. pseudoflavus*	*L. flavus*	*L. maximus*	*L. marginatus*
Open rock face (7)	7	0	0	0	0
Field wall (7)	4	3	0	0	0
Field wall and trees (17)	1	9	0	4	14
Isolated building (9)	6	3	0	1	1
Building and trees (10)	1	8	1	2	6
Town wall (no garden) (5)	0	5	2	2	1
Town walls with garden (2)	0	2	1	2	0
Wall in woodland (6)	0	6	0	1	5
Woodland (4)	0	4	1	3	4
All sites (67)	19	40	5	15	31

TABLE 2. The percentage of each species found in the areas of the Cranagh site between June and November 1978. Analysis of the frequency of occurrence of each species in each habitat in a 4 × 4 contingency table showed there to be significant differences in distribution ($\chi^2 = 19.2$, d.f. = 9, $p < 0.001$). Significance levels in the table refer to *a posteriori* binomial tests performed comparing the observed frequencies with those expected from a consideration of the whole table in order to partition the contingency between individual cells (Siegel, 1956; Stephenson & Poole, 1976). Where significant results were obtained it is also indicated whether the observed frequency of a species constituted a high or a low proportion of the slugs in that subdivision of the habitat. * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$.

Habitat feature	Species			
	L. pseudoflavus	*L. flavus*	*L. maximus*	*L. marginatus*
Ground	23.5	16	35.4** high	7.3*** low
Trees	20.6** high	3.2* low	1.0*** low	30.9*** high
Wall	42.9	74.2*** high	32.5** low	46.4
Roof and wood	12.9* low	6.5** low	31.1*** high	15.5

19.3 ± 3.0 eggs. Eggs were kept moist on filter paper on a bed of plaster of Paris which was continuously in contact with water. The temperatures of the ovens normally deviated from that set by less than one degree.

An egg was deemed viable if an active embryo was visible approximately half way through the incubation period. An embryo was deemed viable if it hatched successfully.

RESULTS

General survey of habitats

The areas examined for the presence of *Limax* species were not chosen at random but were selected so that they all contained stone faces and/or trees which were the habitat features known to be associated with *Limax* species. Table 1 shows a classification of the areas examined and the numbers of those areas which were occupied by a particular species. *L. marginatus* was significantly associated with sites containing trees (chi squared = 16.3; d.f. = 1; $p < 0.01$) but no other significant associations were apparent.

Sites of activity

In a pilot study the distance between the sites of release and collection of freeze branded slugs was measured. 39 *L. pseudoflavus* were marked with four distinctive

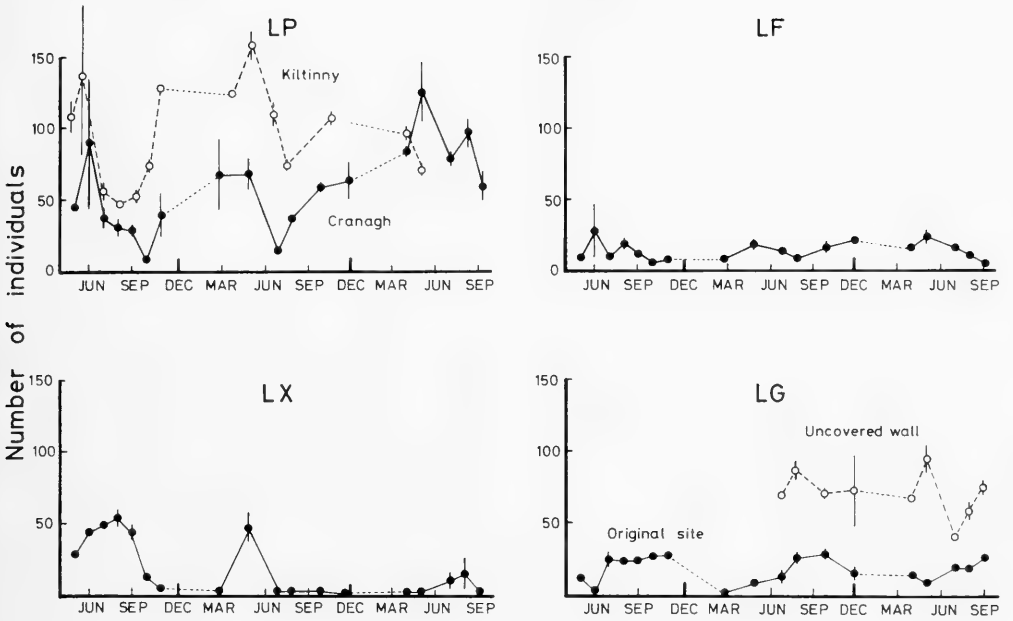

FIG. 2. Estimates (mean ± 95% confidence limits) of the total population sizes at the Cranagh site of LP) *L. pseudoflavus*, LF) *L. flavus*, LX) *L. maximus*, and LG) *L. marginatus*. The data for *L. marginatus* show the population on the original Cranagh site and the wall uncovered in June 1979 and those for *L. pseudoflavus* include both the Cranagh and Kiltinny sites. Sampling commenced in May 1978 and ended in September 1980.

brands and released at four different release points close to their points of collection. On the 22 nights following release only two unmarked individuals were found and no marked slugs were found in the neighbouring Kiltinny site 30 m away. This indicates that immigration and emigration is minimal in the isolated sites being considered. On day 18 the highest number of recoveries was recorded (14) and these were at a mean (± s.e.) distance of 3.2 ± 0.5 m from the release points. Only 2 slugs were found emerging from their original release points. *L. pseudoflavus* therefore does not remain stationary within this habitat.

Fig. 1 shows the details of part of the Cranagh site. The physical features of the area may be divided into 1, the ground in front of the wall, 2, the trees adjacent to the wall (sycamores, *Acer pseudoplatanus*), 3, the wall itself, and 4, the roof which was incomplete and consisted of rotting timbers partially covered with roofing felt. Table 2 shows the frequency with which the four species were found in these sub-divisions of the habitat. It is clear that the species show significant differences in their occupancy of the area. *L. pseudoflavus* and *L. marginatus* constituted a larger proportion of the slugs on the trees than expected, a high proportion of the slugs on the wall were *L. flavus*, and *L. maximus* was found more frequently on the ground and on the roof than the other species. Within this single site therefore the species are not equally distributed over areas with different characteristics.

The frequency and size distribution of field populations

The estimated total populations of each species at both the Kiltinny and the Cranagh site are shown in Fig. 2. For most collections over 80% of the estimated total populations was removed from the site. *L. pseudoflavus* was clearly the most numerous and *L. flavus* the most scarce. *L. maximus* populations decreased from 44 in May to 11 in June 1979 and remained low. This coincides with the clearing of the ground in front of the wall.

The distributions of body weight are shown in Fig. 3 for the Kiltinny site and Figs. 4 to 7 for the Cranagh site. All four species show seasonal changes in population structure, mak-

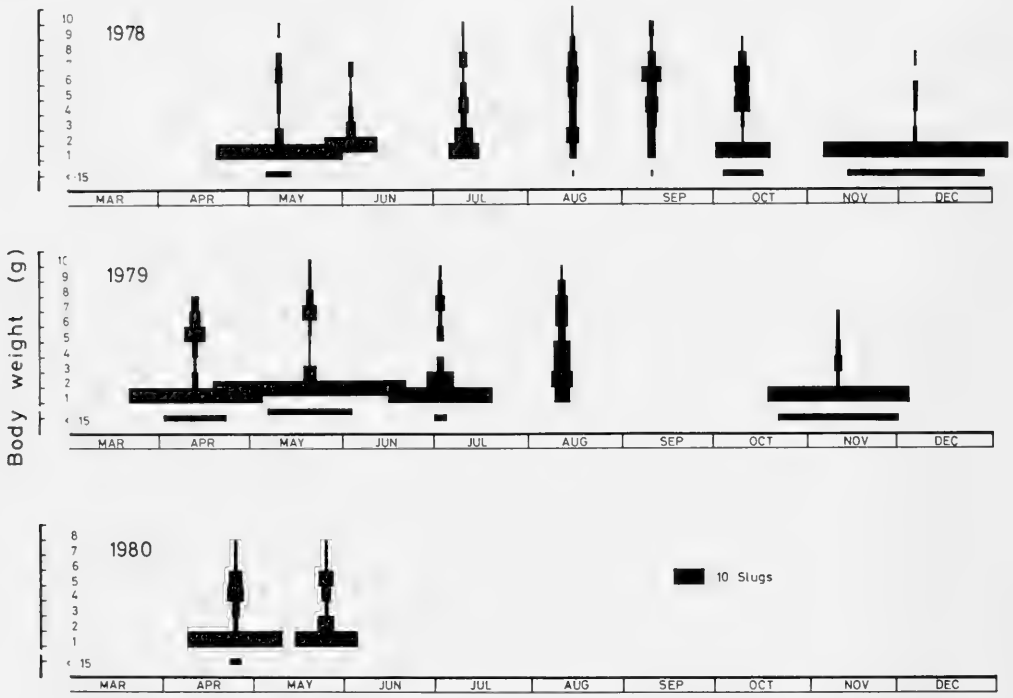

FIG. 3. Size distribution of *L. pseudoflavus* at the Kiltinny site expressed in 1 g size classes. Those slugs of less than 0.15 g in the 0 to 1 g class are also shown separately as this size group reflects the presence of hatchlings in the population.

ing it possible to follow the progress of a generation for most of the first year after hatching.

Small *L. pseudoflavus* appeared in autumn and winter and grew through the spring and summer so that their size distribution merged with that of the smaller individuals of the previous generation (Figs. 3-4). Slugs of less than 1 g formed a greater proportion of the total population at the Kiltinny site (92% in December 1978, and 87% in November 1979) than at the Cranagh (55% in December 1978, and 73% in December 1979). This is a significant difference (e.g. for December 1978, chi squared = 25.33, d.f. = 1, $p < 0.001$).

The *L. flavus* population showed seasonal fluctuations similar to those of *L. pseudoflavus* (Fig. 5). The largest *L. flavus* only attained approximately three-quarters of the size of the largest *L. pseudoflavus*. This is in contrast to their growth in laboratory cultures where *L. flavus* was consistently larger (personal observations DJR).

Although there is a pronounced seasonal pattern in the population structure of *L. maximus* for the early samples the habitat changes at the original Cranagh site in May 1979 makes a full interpretation difficult (Fig. 6). The clearing of the rubbish tip and the general tidying up preceded a reduction in the numbers of *L. maximus* which was not seen in any of the other species, (Fig. 2) and it seems likely that this species had day time resting sites in the rubbish.

There was a difference between the population structures for *L. marginatus* at the original Cranagh site and the uncovered wall (Fig. 7). At the original site the individuals attained a larger size but there were fewer young slugs. In December 1979, 94% of all individuals on the uncovered wall weighed less than 1 g, compared with only 15% in this size class at the original Cranagh site. This difference in size distribution is significant (chi squared = 28.07, d.f. = 1, $p < 0.001$). Data from both sites (Fig. 7) suggest that *L. marginatus* is an annual species, with the eggs hatching in the autumn and winter and very few adults surviving into the following spring. This is particularly clear in 1978 when at the original

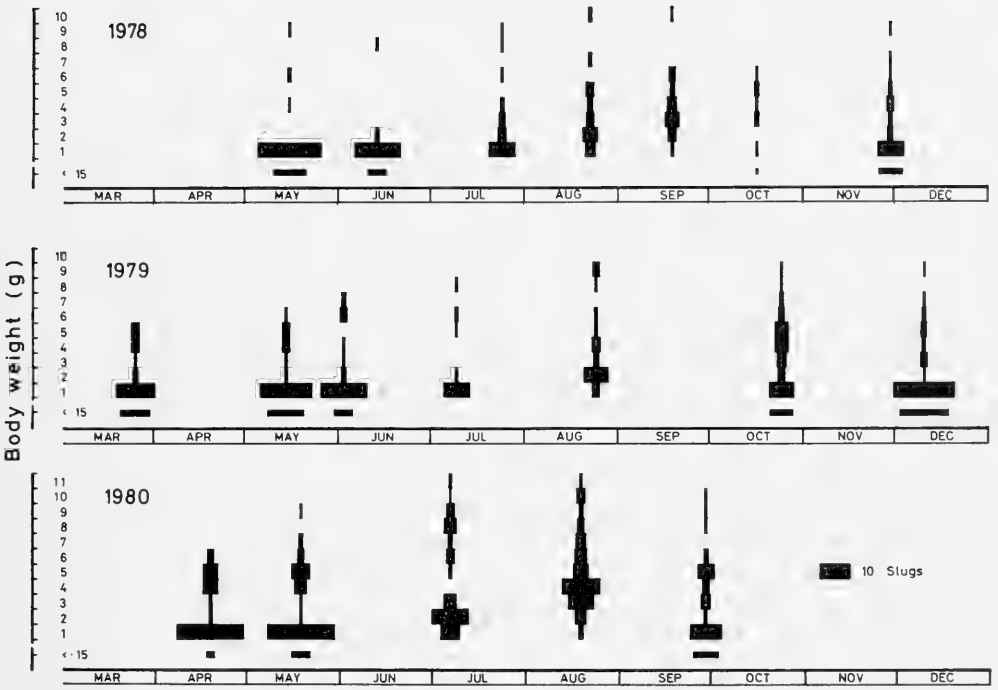

FIG. 4. Size distribution of *L. pseudoflavus* at the Cranagh site. The presence of hatchlings is indicated as in Fig. 3.

Cranagh site the 3, 4 & 5 g slugs decrease in number from a peak of 21 in August to 2 in November to be replaced by new recruitment in the following spring.

Analysis of faecal pellets

There were some significant differences between the months for some species and some food types. They occurred, however for those food items which constituted only small proportions of the faeces and showed no consistent pattern. For ease of analysis therefore, and because too few data were available for some species in some months, further analysis was conducted without regard to the month in which the sample was collected. Table 3 shows the percentage composition of the faeces for each species. Analyses of variance on arc sine square root transformed data showed that there were significant differences between species for all food types except filamentous algae (4). Student-Newman-Keuls *a posteriori* tests (Sokal & Rohlf, 1969) showed that for food types 1, 2 and 3 there were significant differences between *L. flavus* and *L. maximus* and all other species but that there were no significant differences between *L. pseudoflavus* and *L. marginatus*.

Feeding observations

For those individuals found feeding, the substrate over which they were grazing was classified into corticolous lichen (i.e. growing on bark), saxicolous lichen (i.e. growing on the wall), vascular plant, animal, and fungal material. Algae were mingled with the lichen and it is probable that they were consumed together. Table 4 shows the frequency with which the species were found feeding on these classes of substrate. The frequency with which slug species were found on different feeding substrates are as would be expected from a consideration of the sites of activity (Table 2) and the results of faecal analysis (Table 3). Thus *L. pseudoflavus* and *L. flavus* favour saxicolous lichens, *L. maximus* was found feeding on vascular plant material and *L. marginatus* on corticolous lichens. The occasions on which slugs were found feeding on fungi or animal remains were too infrequent to allow further analysis.

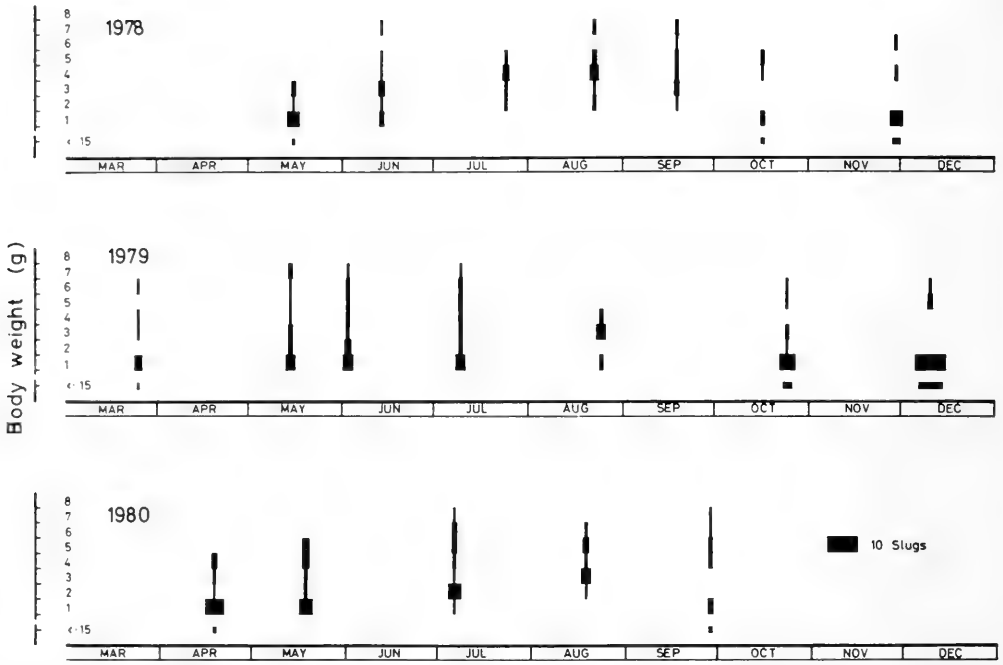

FIG. 5. Size distribution of *L. flavus* at the Cranagh site. The presence of hatchlings is indicated as in Fig. 3.

Niche overlap

Estimation of niche overlap between these species is potentially useful in indicating likely areas of competition although the precise identification of competition depends on the demonstration of resource limitation (Giller, 1984). A measure of Slobodchikoff & Schulz (1980) is concerned with the proportional utilisation of a resource and is therefore, appropriate for this type of data:

$$\text{overlap}_{ik} = 1 - 0.5 \times \sum (p_{ij} - p_{kj})$$

where p_{ij} represents the proportional utilisation of the jth partition of the resource by species i, and the differences between species are summed over all partitions.

This measure of niche overlap based on the comparison of the faecal content (data from Table 3) is given in Table 5. It is clear that the least overlap occurs between *L. maximus* and the other species. Fig. 8 shows niche overlap measured in similar ways for the occupancy of the site (data from Table 2), faecal analysis (data from Table 3) and feeding sites (data from Table 4). These axes are not truly independent since the distribution in the site obviously dictates where the animal may be found feeding and the sites of feeding inevitably bear a relationship to the subsequent faecal analysis. Nevertheless such a presentation serves to illustrate the extent of the differences between species pairs. *L. maximus* is separated from the other species mainly by its feeding habits but also by differences in their occupancy of the site. *L. flavus* and *L. marginatus* are separated largely by their feeding sites. *L. pseudoflavus* is very similar to both *L. marginatus* and *L. flavus* in this particular habitat.

Egg production in laboratory cultures

The number of clutches of eggs and the number of eggs. slug^{-1} month^{-1} are shown in Fig. 9. All species showed a seasonal pattern of egg production. There are differences between the two years for *L. pseudoflavus* and *L. marginatus*. The same *L. pseudoflavus* had been in culture for 18 months by the time their second egg laying period started and both its delay and brevity could be an age effect. The *L. marginatus* died in the spring of the first year and were replaced in the July preceding the

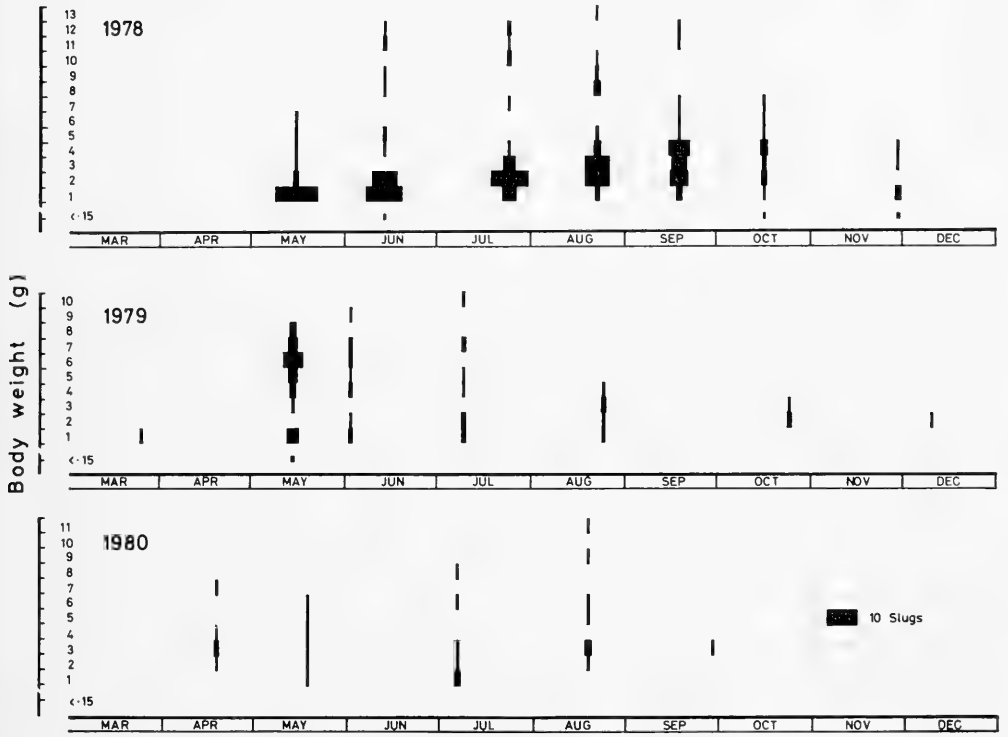

FIG. 6. Size distribution of *L. maximus* at the Cranagh site. The presence of hatchlings is indicated as in Fig. 3.

second egg laying period, the onset of which was delayed.

Influence of temperature on egg development

Comparisons between the viabilities of both eggs and embryos at different temperatures are given in Table 6. Two-way analysis of variance of the transformed egg viability data showed significant main and interaction effects of species and temperature. Student-Neuman-Keuls *a posteriori* testing (Sokal & Rohlf, 1969) of the differences between the means indicates that these effects are largely attributable to the poor survival of *L. flavus* and *L. marginatus* at 5° C and the increased survival of *L. pseudoflavus* at 15° C. Similarly comparisons between the species at the different temperatures indicate that significant effects on embryo viability are attributable to the poor survival of *L. marginatus* at both 15 and 20° C and the higher survival of *L. pseudoflavus* at 5 and 10° C and of *L. marginatus* at 5° C.

The time taken for the first egg to hatch in each batch is shown in Table 7. Again 2 way-analysis of variance showed significant main and interaction effects and these are attributable to significantly shorter development times in *L. maximus* at both 10 and 15° C and significantly longer development times for *L. flavus* at 5° C, *L. marginatus* at 15° C and *L. pseudoflavus* at 20° C. *L. marginatus* failed to hatch at 20° C. The spread of hatching times from single batches of eggs (Table 7) is variable. *L. maximus* shows the greatest range, but since the spread is dependent on the number of survivors further analysis is inappropriate.

DISCUSSION

L. pseudoflavus was the most widespread of the species in the general survey and was the only occupant of 11 of the 67 areas. Experiments in laboratory culture with this species show that it can be kept at high densities without apparent ill effects and that its behaviour in the home is not disrupted by the presence of the other species (Cook,

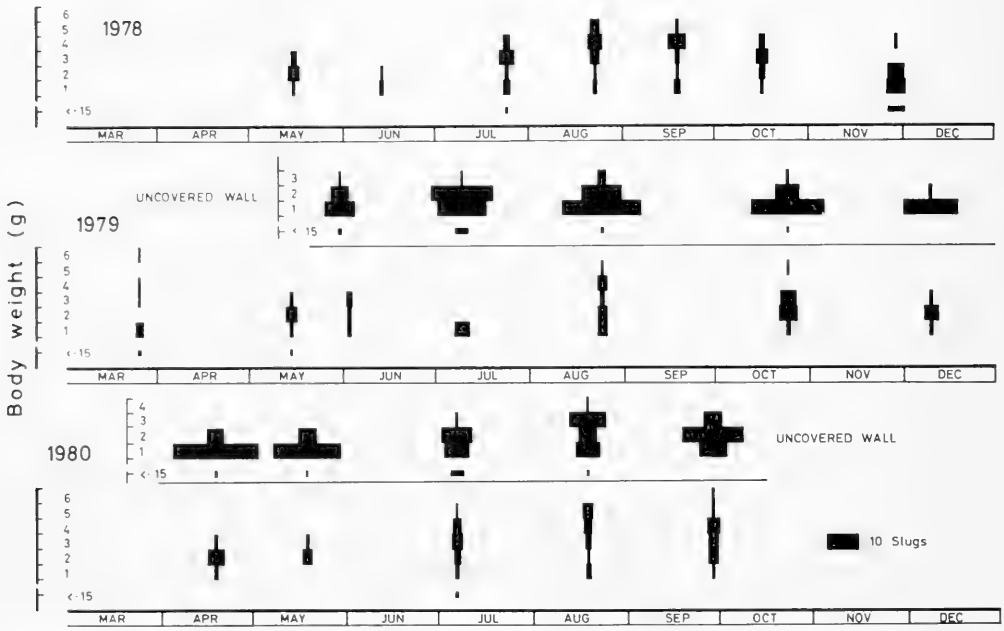

FIG. 7. Size distribution of *L. marginatus* at the Cranagh site and on the 'uncovered wall' (inset). The presence of hatchlings is indicated as in Fig. 3.

TABLE 3. The percentage of each food type found in faecal pellets (mean ± s.e.). n refers to the number of individuals collected from four monthly samples from which pellets were analysed. Analyses of variance followed by Student-Neuman-Keuls tests show that *L. pseudoflavus* and *L. marginatus* did not differ in the frequency in which they took any of the food types. For lichen, vascular plant and *Pleurococcus* all other comparisons showed significant differences. There are no differences between the species for the remaining food types.

Species	Food type					
	Lichen	Vascular plant	Pleurococcus algae	Filamentous algae	Fungi	Mineral
L. pseudoflavus n = 90	62.1 ± 2.4	14.0 ± 2.5	17.2 ± 1.6	0.6 ± 0.3	1.0 ± 0.2	5.2 ± 1.0
L. flavus n = 24	52.1 ± 6.8	30.7 ± 8.0	11.7 ± 3.1	0.1 ± 0.1	0.7 ± 0.3	5.6 ± 1.0
L. maximus n = 38	7.8 ± 3.8	86.3 ± 4.5	0.7 ± 0.4	0.1 ± 0.1	4.3 ± 2.0	0.9 ± 0.3
L. marginatus n = 22	60.5 ± 4.7	15.1 ± 5.1	14.9 ± 3.0	3.0 ± 2.5	3.3 ± 0.8	3.3 ± 0.8

1981b). Within the Cranagh site it was the most common species (Fig. 2), it was significantly associated with trees, but occurred less frequently than expected on the roof compared with other species (Table 2).

L. flavus is very similar to *L. pseudoflavus* in its behaviour (Cook, 1977, 1981a, b). It is the least widespread species in the field, judged by the number of areas at which it is found (Table 1). Four of the five areas at which it was found in the present survey were associated with buildings. The fifth area was a

TABLE 4. The percentage of each species found feeding on different substrates. n refers to the total number of that species which was found feeding. Ignoring animal and fungal material which were present in too few observations to allow analysis, there was a significant deviation from a random expectation when the original frequencies were considered ($\chi^2 = 62.1$, d.f. = 6, $p < 0.001$) Significance levels in the table refer to the results of binomial tests (* = $p < 0.05$, ** = $p < 0.01$, *** $p < 0.001$.) For each significant result an indication is given of whether the frequency is higher or lower than expected from a consideration of the whole table.

Species	Saxicolous lichen	Corticolous lichen	Higher plant material	Animal	Fungi
L. pseudoflavus n = 326	67.5* high	24.8 —	5.8** low	1.8	0
L. flavus n = 71	74.6 —	15.5* low	8.5 —	1.4	0
L. maximus n = 49	18.4*** low	8.2** low	57.1*** high	12.2	4.1
L. marginatus n = 115	46.1* low	48.7*** high	5.2* low	0	0

TABLE 5. Indices of niche overlap based on food consumption inferred from faecal analysis. (Data from Table 3.) A value of 1 would indicate total overlap.

	L. flavus	*L. maximus*	*L. marginatus*
L. pseudoflavus	0.835	0.266	0.949
L. flavus	—	0.428	0.844
L. maximus		—	0.290

garden and had also been considerably influenced by man. Within the Cranagh site it was the least numerous species prior to the disruption in May 1979 (Fig. 2) and found more often than expected on the wall and less often on the trees and roof.

L. marginatus has a similar distribution to *L. pseudoflavus.* It is however, significantly associated with trees, both in those areas in which it occurs (Table 1) and in its position within a single area. At the Cranagh it was rarely found on the ground (Table 2).

L. maximus has a more restricted distribution than either *L. pseudoflavus* or *L. marginatus.* It occurs mostly at sites which also contain trees (Table 1), although at the Cranagh its frequency on the trees was significantly lower than expected. This was also apparent during the general survey in which *L. maximus* was normally found in the litter and among stones and fallen logs rather than actually on trees.

There are significant differences in the sites of activity of these four species but these do not amount to a substantial stratification of the species within the habitat (Table 2). Previous work on the sites of activity of terrestrial molluscs also failed to demonstrate substantial spatial separation of closely related sympatric species (Cameron, 1978).

The growth of young slugs can be followed in the size distributions (Figs. 3 to 7). As slugs become older the size boundaries between cohorts breaks down. This lack of distinction between the cohorts older than about six months is probably a consequence of the highly variable growth rates of slugs (Prior, 1983). Thus it is difficult to draw conclusions concerning the age structure of any but the smallest slugs.

There are clearly at least two generations of *L. pseudoflavus*, *L. flavus* and *L. maximus* present throughout the year and most individuals of these species are capable of breeding in their first autumn (personal observation DJR). They are therefore probably polyvoltine and semelparous. Most large terrestrial gastropods have adopted this type of life cycle (Peake, 1978). Most slugs which have been studied breed in their first year (Runham & Hunter, 1970), though some of the larger polyvoltine shelled species take longer to

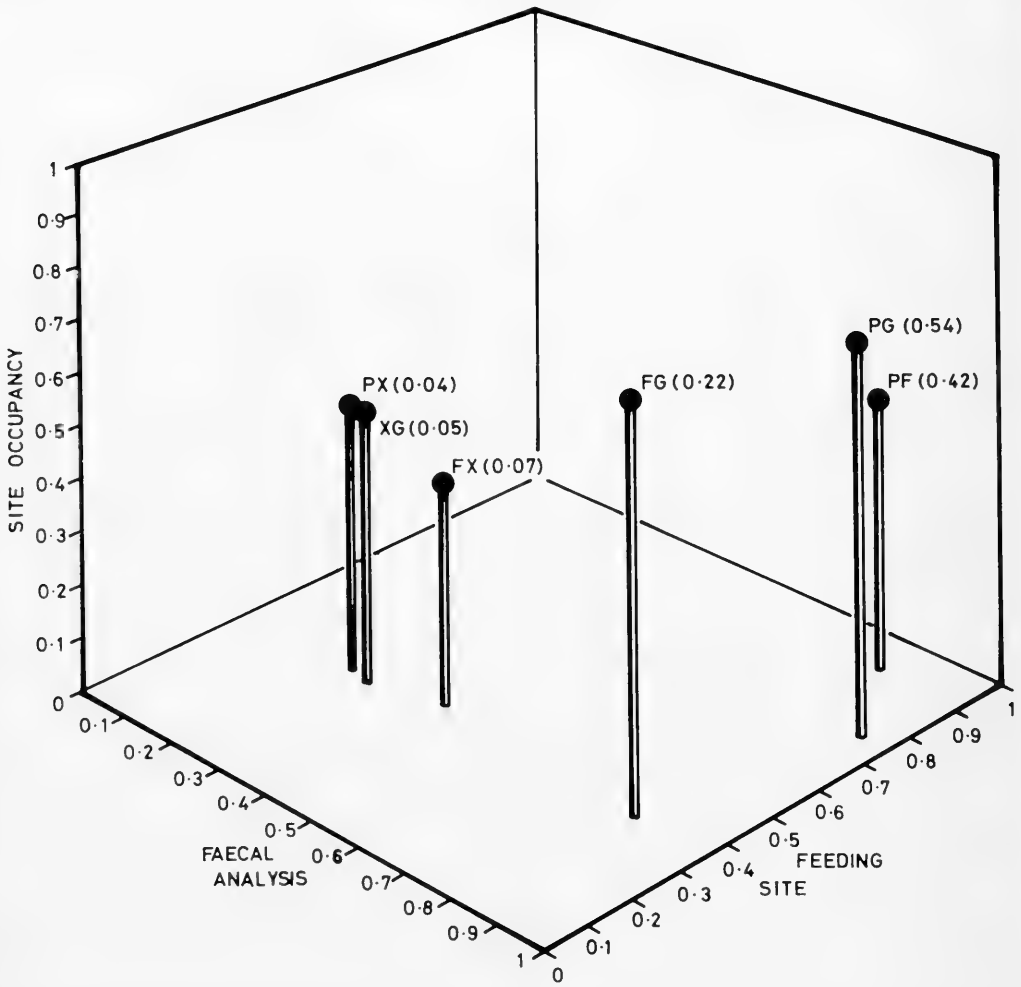

FIG. 8. A three dimensional view of three measures of niche overlap. Each point represents the overlap between one pair of species (e.g. point PG represents the point for *L. pseudoflavus* and *L. marginatus*). The figures in brackets are the products of the three measures of overlap and indicate the proportion of the total space occupied in common by the species concerned. P- *L. pseudoflavus*, F- *L. flavus*, X- *L. maximus*, G- *L. marginatus.*

mature (Cowie, 1984). Most of the large autumn individuals of *L. marginatus* die during the winter and spring to be replaced the following autumn by the previous years hatchlings. It therefore has a univoltine, iteroparous life cycle.

Small *L. pseudoflavus*, *L. flavus* and *L. maximus* (0-0.15 g) were present in the field populations from September to May or June (Figs. 3 to 7). Those small slugs still present in late spring probably hatched a month or more earlier (Fig. 9). Hatching in the field therefore occurred from September to about April. This corresponds reasonably well to the laboratory egg-laying periods though there are some disparities for *L. flavus.*

Small *L. marginatus* (0-0.15 g) were present in the field populations in extremely low numbers throughout the year. This species is the smallest of the four and is an annual, but the prolonged presence of small individuals in the field (Fig. 7), together with the well defined egg-laying period (Fig. 9) have no obvious interpretation.

Comparison of slugs from sites occupied by all four species with those from sites occupied by only one is potentially useful in identifying changes brought about by coexistence. Infor-

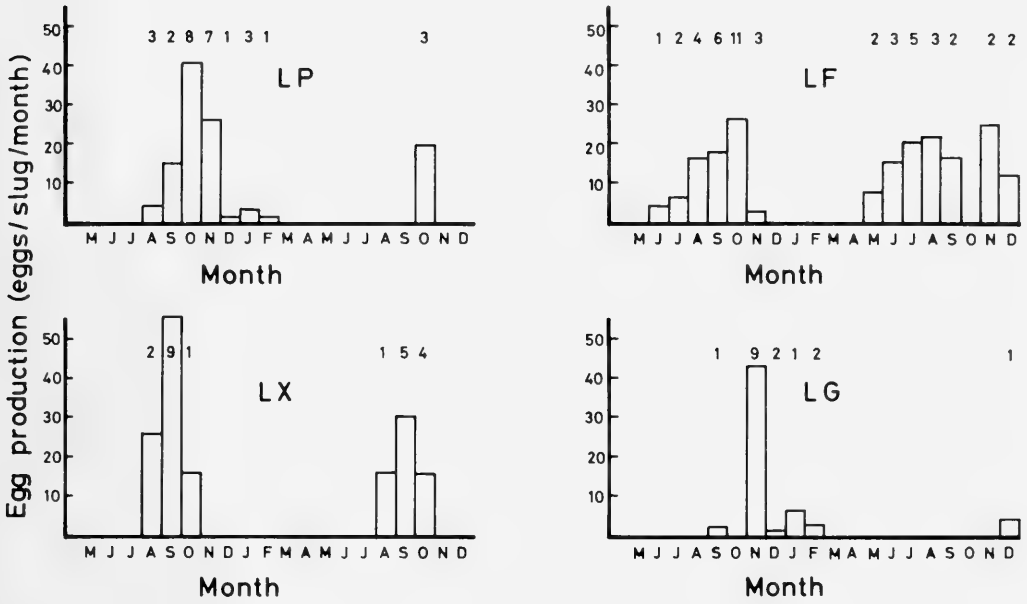

FIG. 9. The egg production of slugs in culture (egg/slug/month). The numbers refer to the number of clutches of eggs laid per month. LP) *L. pseudoflavus*, LF) *L. flavus*, LX) *L. maximus*, LG) *L. marginatus*.

mation has been presented for the size distribution for *L. pseudoflavus* and *L. marginatus* at such sites and for both species there was a significant increase in the proportion of small animals in populations where other species were not present. These differences in size distribution may be attributable to a variety of factors which may not necessarily be associated with the presence or absence of other *Limax* species. *Limax* species are known to share day-time resting sites, at least under laboratory conditions (Cook, 1981b) despite exhibiting some interspecific aggression (Rollo & Wellington, 1979). The significance of disturbances in the home brought about by a second species with different requirements is unknown though it has been suggested that aggressive interactions between slugs may reduce the energy available for growth (South, 1982).

The faecal string of slugs consists mostly of those items of food too large to be passed to the digestive gland for intracellular digestion and can therefore be used to indicate the substrate over which the animals had been feeding. Faecal analysis however does not give precise details of diet since only ingested items rejected by the stomach can be identified. The analysis of the faeces shows that, unlike the other species *L. maximus* feeds predominantly on vascular plant material. This correlates with the frequency with which it was found grazing on wood. The sites of activity of this species (Table 2) show a significant preference for the roof and the ground both of which would provide rotting vascular plant material. The lack of mineral material in the faeces is further evidence that few *L. maximus* feed on the wall. About 27% of the *L. maximus* which were found feeding were grazing on lichens whereas only 8% of the faecal material was lichen. This discrepancy may arise because the animals were collected whilst feeding on their way to normal grazing areas rather than actually at them and suggests that the animals on the roof had travelled there from resting sites on the ground.

The sensitivity of the eggs of *L. flavus* to low temperatures, its comparative scarcity in field communities (Fig. 2 & Table 1) and the observation that Northern Ireland is near the northern limit of its European distribution (Kerney & Cameron, 1979) support the view of Bruijns *et al.* (1959) that this species is of Mediterranean origin. *L. marginatus* on the other hand has the most northerly distribution of the species under consideration, being found in Iceland and on the coast of northern Norway (Kerney & Cameron, 1979). The comparatively high viability of its embryos at

TABLE 6. A comparison of the survival of eggs and embryos incubated at different temperatures. Egg viability (% of eggs producing a normal embryo) and embryo viability (% of normal embryos successfully hatching) are shown separately. 2 way analysis of variance on arc sin square root transformed data for egg viability showed both main effects (Temperature, $F = 11\ 35$; d.f. = 3,99; $p < 0.001$: Species, $F = 6.61$; d.f. = 3,99; $p < 0.001$) and the 2-way interaction (Species × temperature, $F = 3.21$; d.f. 9,99; $p < 0.002$) to be significant. A similar analysis for embryo viability showed again that both main effects (Temperature, $F = 17.94$; d.f. = 3,90; $p < 0.001$: Species, $F = 7.49$; d.f. = 3,90; $p < 0.001$) and the 2-way interaction (Species × temperature, $F = 4.23$; d.f. = 9,90; $p < 0.001$) were significant.

	Incubation temperature			
	5°C % viable	10°C % viable	15°C % viable	20°C % viable
L. pseudoflavus				
egg	82.5 ± 0.6	87.8 ± 0.3	96.0 ± 0.3	77.9 ± 0.9
embryo	78.1 ± 1.5	99.5 ± 0.2	99.4 ± 0.3	82.2 ± 0.7
L. flavus				
egg	16.2 ± 2.1	76.6 ± 2.1	83.6 ± 0.1	85.5 ± 0.2
embryo	17.3 ± 8.5	91.7 ± 0.9	97.6 ± 0.5	92.4 ± 0.6
L. maximus				
egg	79.4 ± 2.1	90.5 ± 1.1	83.6 ± 0.7	88.6 ± 0.2
embryo	16.4 ± 6.5	77.4 ± 2.7	98.9 ± 0.2	83.2 ± 0.2
L. marginatus				
egg	54.4 ± 3.3	86.8 ± 15.4	71.9 ± 1.6	63.9
embryo	82.7 ± 9.2	67.6 ± 1.7	62.9 ± 1.6	0.0 ± 0

TABLE 7. The time in days to the hatching of the first egg in a batch (mean ± s.e.) and the duration in days of the hatching period (mean ± s.e.) A 2-way analysis of variance of the hatching times showed there to be significant main effects (Temperature – $F = 1003.59$; d.f. = 3,82; $p < 0.001$: Species – $F = 6.96$; d.f. = 3,82; $p < 0.001$) and 2-way interaction (Species × temperature – $F = 7.92$; d.f. = 8,82; $p < 0.001$).

	Temperature			
Species	5° C	10° C	15° C	20° C
L. pseudoflavus				
hatching time	146 ± 4.7	63 ± 2.1	32 ± 0.3	26 ± 1.7
duration	13.9 ± 3.4	4.0 ± 1.2	2.9 ± 0.4	4.3 ± 2.9
L. flavus				
hatching time	183 ± 7.8	67 ± 1.8	32 ± 0.3	23 ± 0.4
duration	18.0 ± 18	13.8 ± 3.4	4.0 ± 1.0	3.6 ± 1.1
L. maximus				
hatching time	138 ± 10.9	56 ± 1.0	28 ± 0.5	20 ± 0.7
duration	43.5 ± 14.5	20.8 ± 6.9	5.1 ± 3.0	8.7 ± 3.7
L. marginatus				
hatching time	141 ± 10.8	64 ± 1.3	45 ± 2.1	—
duration	25.7 ± 7.2	11.8 ± 2.4	2.7 ± 1.2	—

5° C and the low viability at 20° C (Table 6) correlates well with this distribution.

The period over which an egg batch hatches clearly varies with temperature (Table 7) but within a species there is little difference when the duration of the hatching period is considered as a proportion of the time taken for the first egg of a batch to hatch. *L. maximus* showed the greatest duration of the hatching period at all temperatures despite having the shortest incubation time. This is surprising since it has been reported by Prior (1983) that single clutches of eggs of *L. maximus* all hatch on the same day.

TABLE 8. A summary of the differences between *Limax* species. Additional data from Cook (1981b ') and Kerney & Cameron (1979*).

Factor	*L. pseudoflavus*	*L. flavus*	*L. maximus*	*L. marginatus*
Predominant habitat type	Wall/trees	Walls	ground	trees
Predominant food type	saxicolous lichen	saxicolous lichen	vascular plant	corticolous lichen
Life cycle type	polyvoltine semelparous	polyvoltine semelparous	polyvoltine semelparous	univoltine iteroparous
Temperature sensitivity of embryo				
Lowest mortality	15,10° C	10,15,20° C	10,15,20° C	5° C
Highest mortality	5,20° C	5° C	5° C	15,20° C
Northern limit to distribution*	?	Scotland/Denmark	S. Norway/Sweden	Iceland/N. Norway
Peak egg-laying period	Aug-Nov	Jun-Dec	Aug-Oct	Nov-Feb
Distribution in home+	huddled	huddled	touching	dispersed

Whilst there are major differences in the sensitivity of these species to temperature which may be related to their geographical distribution, their broad egg-laying strategy is similar. Egg laying in autumn and early winter reduces the dangers from dehydration and predation by insects both to the eggs and the juveniles. Furthermore, egg-laying at this time allows slugs to overwinter as eggs, juveniles or adults giving a potentially flexible response to the varying conditions of winter.

These four species of *Limax* are obviously different in morphology, but this and other work (Cook, 1981b) have shown substantial ecological and behavioural differences. These differences are summarised in Table 8.

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INCOMPLETE CONVERGENCE OF SHELL SIZES AND SHAPES IN FOREST SNAIL FAUNAS FROM TWO CONTINENTS: A RELIC OF ENVIRONMENTAL HISTORY?

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ABSTRACT

A comparison is made of the range of shell sizes and shapes in forest snail faunas from British Columbia and north-west Europe. While there are many species in each region which share characters of size and shape with species in the other, there are also differences.

British Columbian faunas lack large tall-spired species, and have fewer flattened or globular species of medium or large size than those of Europe. Conversely, they have more very small species. While there are a few cases of close convergence, there are also species in each region which differ substantially from any found in the other.

This non-convergence cannot be accounted for solely as a product of present environmental differences between the regions, either in climate or in vegetation. An explanation is offered in terms of the effects of on the Pleistocene and Holocene history of the two regions, and in particular on the opportunities for speciation and colonization presented. The absence of appropriately shaped ancestors in source areas for colonization after retreat of Pleistocene ice-sheets may be of particular importance.

INTRODUCTION

Ecological and evolutionary theories predict that when similar ecological niches are occupied, in different regions, by different species, those species will show similarities in those aspects of their morphology that relate to the niche occupied. Where such similarities cannot be accounted for by common ancestry, they are examples of convergent evolution (Cain, 1964). In particular cases, convergence may be remarkably exact, and the species concerned are referred to as ecological equivalents (e.g. Cox & Moore, 1973).

Where such convergences are numerous, the situation provides strong evidence for the operation of natural selection (Cain, 1964). Where convergence does not occur, however, a variety of explanations are possible, and are not mutually exclusive. Assumptions concerning the similarity of niches may be wrong; the morphological characters may not relate to the aspects of the niches which are similar; time elapsed since the occupation of the niche may be too short for convergence to be complete, or developmental constraints or adaptive troughs (Wright, 1932) may prevent convergence or delay its completion.

This study compares certain features of shell morphology in terrestrial snail faunas from forests in coastal British Columbia and in north-west Europe. These regions experience very similar temperate and oceanic climates. Temperature regimes are very similar, January means varying from 0-6° C and July means from 13–19° C—figures varying between individual stations, but with complete overlap between regions (Anon., 1982a, 1984). The range of precipitation is great in both regions (700 mm–2000 mm + per year), but rainfall is more seasonal in coastal British Columbia, much more falling in winter than summer (Anon., 1982b, 1984, Waring & Franklin, 1979). Nevertheless, many European sites have summer rainfall of comparable magnitude to British Columbian sites.

The range of forest soils and litter is also comparable, with podsolic soils and mor litter in poorer sites, and brown earths and mull litter in the richer ones (Klinka, Green, Trowbridge & Low, 1981). The close similarity of abiotic conditions generally in the two regions is confirmed by the practical experience of the British Forestry Commission; after much experimentation, plantings of non-native conifers in Britain are predominantly of species and stocks from the Pacific North-West, and particularly from the coastal region.

These perform better than stocks and species derived from more continental climates (Locke, 1970).

Forests in coastal British Columbia are predominantly coniferous and evergreen. In N.W. Europe, such forests tend to occur in montane or high latitude zones climatically more extreme than the lowlands, which are dominated by deciduous broadleaves. Coniferous forests tend to be associated with, and to produce acidic soils with mor litter, a combination hostile to terrestrial snails. This association is not, however, complete; British Columbian conifer forests with brown earth soils and mull litter do exist. Furthermore, some British Columbian forests are dominated by broadleaf deciduous trees, and their snail faunas do not differ significantly from those of conifer forests with comparable soil and litter conditions (Cameron, 1986). Both in N. America and in Europe, conifer forests with appropriate soil and litter conditions have snail faunas comparable to those of deciduous forests, as demonstrated in Cameron (1986).

Snail faunas from forests in both regions have been sampled in similar ways. They share some families, genera and species, but show sufficient taxonomic differences to make the comparison interesting. Features of morphology considered are those for which there is evidence for their adaptive significance.

MATERIAL AND CHARACTERS USED

Data on the composition of snail faunas from forests in coastal British Columbia are taken from Cameron (1986), and are based on 38 sample sites. One alien species, *Vallonia pulchella*, found at one site, has been omitted from consideration.

For European comparisons, the results of three studies are used. That of Cameron (1973), based on 44 sample sites in deciduous broadleaf forests on the South Downs, S. England, uses identical sampling techniques. That of Körnig (1966) covers a very wide range of habitats in central Germany; the rich *Hangbuchenwalder* series (20 sites), also from deciduous broadleaf forests is used here.

British Columbian forests tend to be dominated by evergreen conifers, even on limestone and on mull litter. The third European study used is that of Schmid (1966) from the Spitzberg, W. Germany, using 42 samples made in spruce (*Picea*) and fir (*Abies*) forests on favourable soils. Other quantitative European studies in coniferous forests are not on soil and litter types comparable with the richest Canadian sites, and have impoverished faunas (see discussion in Cameron, 1986).

The British Columbian, English and German *Hangbuchenwalder* samples were made by a combination of searching and litter sampling and sieving, and give comparable estimates of frequencies.

Schmid's survey differs from these in one important respect; each sample represents the sieving and searching of 1 m^2 of litter, and frequency data are not comparable with those from the other studies, as larger species in particular will have lower frequencies per sample, and some may be missed altogether.

The appendix lists the species recorded in each study, and their frequency of occurrence in the British Columbian, British and the German *Hangbuchenwalder* samples. Nomenclature for the European sites follows Kerney & Cameron (1979), and for British Columbia Branson (1977) with a few exceptions noted in Cameron (1986).

The principal characters used in this study are the height and maximum diameter of the adult shell. For European species, the data were obtained from Kerney and Cameron (1979), using mid-points where a range is given. For British Columbian species, the data come from measurements of adult shells collected by Cameron (1986), except in the cases of *Euconulus fulvus* and *Zonitoides arboreus*, where no shell measured approached the dimensions given by Pilsbry (1939-1948). His data are used for these species; in all the rest, differences between his data and those obtained by measurement of Cameron's material are very slight. Data on number of whorls, and on the rate of whorl expansion come from the same sources.

RESULTS

Fig. 1 shows logarithmic plots of shell height and diameter for the species recorded in each study. Dashed diagonal lines indicate contours of approximate volume, derived from actual measurements of weight in European species (Cameron, 1981), supplemented by estimating volumes of cones with specified heights and basal diameters.

The range of diameters, and of volume is

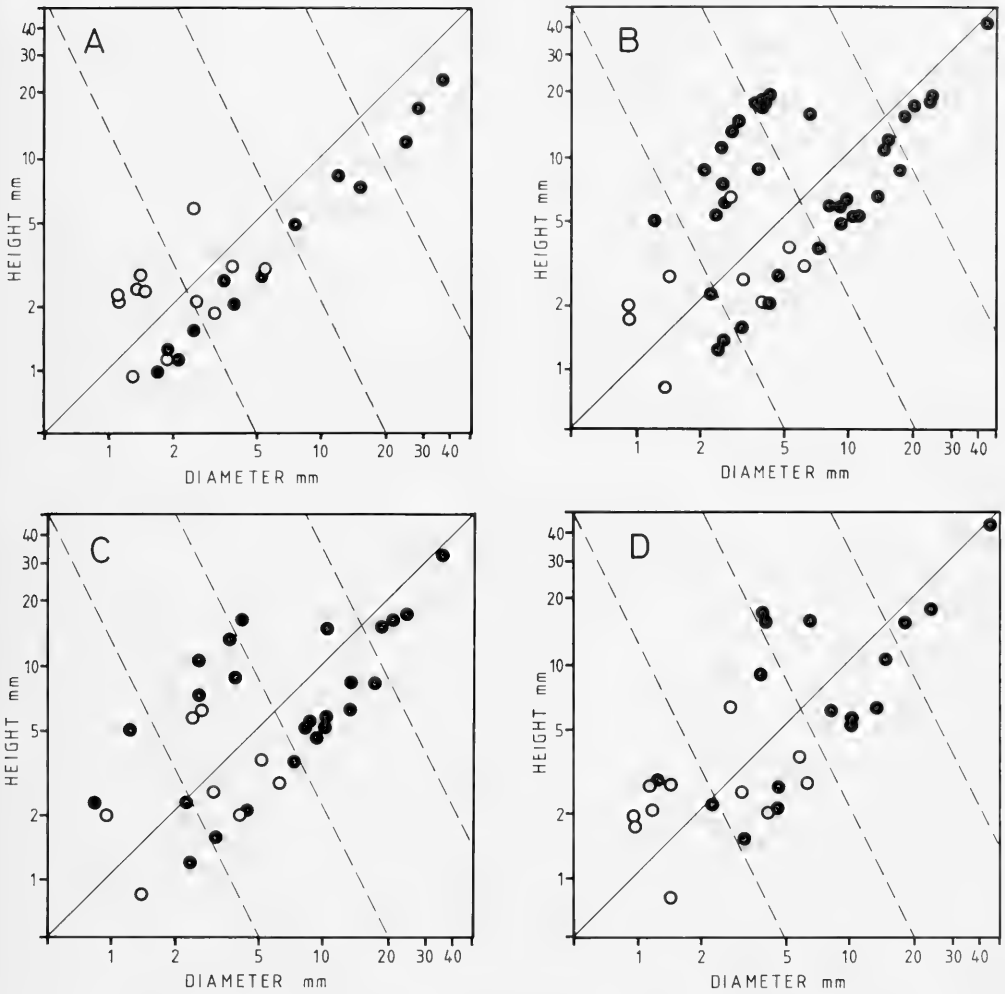

FIG. 1. Logarithmic plots of shell height and diameter for (A) species in British Columbian forests, (B) species in beechwoods in central Germany, (C) species in beechwoods in S. England, (D) species in west German conifer forests. Open circles represent species in genera common to both British Columbian and European sites. The bisector is the line of equal height and diameter. Dashed lines represent contours of volume: from left to right, 7 mm^3, 100 mm^3, 1400 mm^3.

similar in all scatters, which also shows the characteristic bimodality in height/diameter ratios described by Cain (1977).

There are, however, differences between the regions in the proportion of species occurring in different parts of the scatters. In particular, larger tall-spired species are missing in British Columbia, where only one species with a tall spire has a volume greater than 7 mm^3.

Other differences also occur (Table 1a). The British Columbian fauna has more very small flattened/globular species and fewer large species of the same shape than any of the European faunas.

Species in genera common to the faunas in both continents are shown with open circles in figure 1. Since similarities between them might reflect common ancestry, table 1b shows the effect of removing them from the size/shape comparisons. Although their removal further reduces the already limited number of species involved, the trends noted above persist, and in some cases intensify.

Two trivial phenomena could complicate

TABLE 1. Numbers of species classified by size and shape of shell (a) for all species, (b) excluding species in genera common to both Europe and British Columbia. See text for sources of data.

	Tall-spired species			Flattened and globular species			
Volume:	< 7 mm^3	7–100 mm^3	> 100 mm^3	< 7 mm^3	7–100 mm^3	> 100 mm^3	Total
(a)							
British Columbia	5	1	0	6	7	6	25
South Downs	3	6	2	3	7	13	34
C. Germany	4	9	6	4	8	15	46
W. Germany (conifers)	6	2	3	2	7	8	28
(b)							
British Columbia	0	0	0	4	3	6	13
South Downs	2	4	2	2	3	13	26
C. Germany	1	8	6	3	4	15	37
W. Germany (conifers)	1	1	3	1	3	8	17

TABLE 2. Mean numbers of species per site classified by size and shape for 3 comparable studies.

	< 7 mm^3	7–100 mm^3	> 100 mm^3
Tall-spired species			
British Columbia	2.6	0.2	0
South Downs	1.9	3.7	2.0
C. Germany	0.9	3.7	2.9
Flattened and globular species			
British Columbia	4.5	2.8	4.4
South Downs	1.8	3.9	6.1
C. Germany	1.8	3.6	9.0

the interpretation of these trends. Replacement of some species by others of similar size and shape in various samples from the same region would inflate the number of species in that size and shape class relative to a region in which the same species were present in all samples. A similar bias could occur if rare or accidental species, occurring in only one or two sites were commoner in one region than in another.

The effects of these phenomena can be removed by weighting each species by the frequency of its occurrence, a procedure which gives, in effect, the number of species of any given size and shape that one would expect to find in a single sample. Schmid's (1966) data for German coniferous woods are not comparable with others and have not been used. In both the English and British Columbian surveys the sites studied include some with acid soils and impoverished faunas, whereas the *Hangbuchenwalder* series of Körnig is ecologically uniform. To allow for this, frequencies of occurrence in England and British Columbia are based on the richest association-type found: the group C sites ($n = 24$) of Cameron (1973), and the mull series ($n = 19$) of Cameron (1986).

Table 2 shows that differences between the British Columbian and European faunas are maintained when frequencies are used.

Other similarities and differences between the shells of species in the two regions are more subtle, and are best considered in comparisons between species of similar size, shape and habit.

Amongst very large species (volume 1400 mm^3 +), there are no very precise convergences (Table 3a). The European species (helicids and one bradybaenid) are all more globular, and have more rapidly expanding whorls relative to their size (Cameron, 1981) than British Columbian *M. fidelis* and *A. townsendiana* which also differ from the helicids in being umbilicate, and in having more whorls. The closest match, other

TABLE 3. Shell characters of (a) species larger than 1400 mm^3, (b) species showing close inter-continental resemblances between 100 and 1400 mm^3. H/D = height to diameter ratio; Whorls = number of whorls to nearest 0.25 whorl; Umbilicus = width of umbilicus relative to diameter, Raup's W = rate of whorl expansion. See Cameron (1981) for details of measurement.

(a) Very large species

	mm Diameter	H/D	Whorls	Umbilicus	Raup's W
Europe					
Arianta arbustorum	21	0.76	5.5	0.02	1.66
Bradybaena fruticum	20	0.85	6	0.11	1.80
Cepaea hortensis	18	0.83	5.25	0	1.72
Cepaea nemoralis	24	0.76	5.5	0	1.72
Helix aspersa	36	0.89	5	0	2.09
Helix pomatia	45	0.95	5.5	0	2.16
British Columbia					
Monadenia fidelis	36	0.64	6.5	0.08	1.56
Allogona townsendiana	27	0.62	5.75	0.09	1.64
Haplotrema vancouverense	24	0.48	5.25	0.20	1.94

(b) Medium-to-large species—closest comparisons only

	mm Diameter	H/D	Whorls	Umbilicus	Raup's W	Hairs	Barriers
Europe							
Isognomostoma isognomostoma	9	0.61	5.5	0	1.38	yes	yes
Perforatella incarnata	14	0.69	6	0.09	1.40	no	no
Trichia hispida	8	0.63	6	0.20	1.41	yes	no
Trichia plebeia	8	0.69	5.5	0.13	1.45	yes	no
Trichia striolata	13	0.61	6	0.17	1.53	juv.	no
British Columbia							
Vespericola columbiana	13	0.67	5.25	0.05	1.44	yes	no
Triodopsis germana	7	0.65	5	0.05	1.32	yes	yes

than in size, is between *Arianta arbustorum* and *Allogona townsendiana*, a match which extends to shell pattern and colour. All these species have in common a relatively thick shell, and an everted or thickened peristome. The other large British Columbian snail, *H. vancouverense*, has no north- west European equivalent—it is very flattened, has a thin, horny shell, a large umbilicus and a simple peristome. In these features, as in its reputedly carnivorous habits, it resembles the zonitids of north-west Europe, but is far larger than any of them.

Amongst somewhat smaller species (100-1400 mm^3) which are globular or flattened, the European fauna is more diverse both in taxa and in the range of shell characters. Of the three British Columbian species, *H. sportella* has some resemblances to zonitids, but has a thicker, sculptured shell and an everted peristome. The two polygyrids, *T. germana* and *V. columbiana* do show rather close resemblances to a number of helicids (Table 3b), but there are others which lack British Columbian equivalents, such as the sharply keeled *Helicigona lapicida.*

There are fewer species in the smaller size classes which lack congeners on the other continent. European *Vitrea* species show some resemblances to North American *Microphysula*, and, to a lesser extent to *Pristiloma*, which, while shiny and tightly coiled, are brown, and have an appreciable spire. The minute British Columbian *Planogyra clappi* and *Striatura pugetensis* have no obvious European equivalents; both expand their whorls more rapidly, and have larger umbilicuses than either European or N. American *Punctum* species.

All tall-spired British Columbian species

have congeners in north-west Europe, and congeners resemble each other very closely. The larger tall-spired European snails belong to families not present in N. America.

DISCUSSION

Convergence or parallelism for the characters studied in these British Columbian and European snail faunas is far from complete. There are some convincing convergences between distantly-related species, and other similarities which could have derived from common ancestry. There are also differences between the faunas, both containing species for which there is no obvious equivalent in the other.

The characters studied relate to mode of life in snails. Cain (1977, 1981) has shown that there is a near-universal bimodality in the distribution of height-diameter ratios in terrestrial pulmonate faunas, and studies both in this field and in the laboratory have shown associations between this measure of shape and the preferred angle and type of substrate for activity (Cain & Cowie, 1978, Cameron, 1978, Cook & Jaffar, 1981). High-spired species tend to prefer hard vertical surfaces, such as rock, tree trunks and logs, while more flattened species resort more to horizontal surfaces. Globular shelled species may be better adapted to mobile surfaces (such as living or senescent herbaceous plants). Cain's work, cited above, makes it clear that these repeated patterns of size and shape have arisen independently in faunas of different taxonomic origin. Cameron (1981) discusses the functional significance of some other shell characters used, such as rate of whorl expansion.

In terms of present environment in the two regions, the slight climatic differences seem inadequate as an explanation of these faunal differences. British Columbia does have a more seasonal pattern of precipitation than north-west Europe, but European forest snails of shapes and sizes not found in British Columbia, especially in the families Helicidae, Clausiliidae and Enidae, extend into the Mediterranean region which has hot dry summers. Soil and litter conditions are comparable; all the sample series considered here include sites on limestone derived soils with high pH—the optimum for snail diversity.

Differences in the nature of the predominant forest cover could be of more significance. Much of the literature on forest molluscs (reviewed in Cameron 1986) stresses the comparative poverty of coniferous forest on both continents. This poverty is not, however, simply a product of conifer cover *per se*; it is primarily a consequence of the nutrient-poor and acidic soils, of the sometimes excessively dry and wet conditions, and of the adverse climates on and in which conifer forests are commonly found. Where these conditions are ameliorated, conifer forests can support diverse snail faunas. Thus, in British Columbia, snail faunas from conifer stands in bottomlands or on limestone derived soils do not differ significantly from those from broadleaf stands in the same situation. The example of the Spitzberg, used here, is not the only one of species-rich coniferous forest in Europe. Other, less quantitative studies (summarized in Cameron, 1986) also describe diverse faunas from coniferous forests, including species of sizes and shapes not found in British Columbia. Given the botanical and climatic similarities involved, and the absence of clear distinctions in the faunas of coniferous and broadleaved forests in both regions, the differences seen would not be predicted on a simple *a priori* hypothesis that available niches should be filled.

Direct effects of conifer cover should not, however, be completely excluded. In particular, there might be a connection between the relatively high proportion of very small species of snail and the small average particle size of litter. Such small species (especially Vertiginidae) predominate also in conifer forests in colder climates (e.g. Wareborn, 1969).

If characters considered relate to niche occupied, and present physical and botanical environments do not appear to prescribe a radically different range of potential niches, it would seem that some of the niches are empty. Why might this be so?

One possible explanation might lie in differential risk from predators between regions, rendering certain niches untenable in one region. There is, at present, insufficient evidence to refute or confirm such an hypothesis. Cain's (1977) scatters of height and breadth, based on many faunal regions, show strong representation above and below the bisector in regions of diverse climates and habitats, in which the range of predators presumably differs. Only in rather harsh, cold continental climates does the upper scatter attenuate or disappear (Cain, 1981).

Given the slow active dispersal of snails, and the morphological conservatism shown in many families (Cain 1977, 1981), a part of the

answer might lie in the histories of the regions concerned and the nature of the snail faunas available to colonize them. The environmental history of both regions is heavily influenced by the Pleistocene glaciations. Both were subject to glacial or peri-glacial conditions at the last advance of the Pleistocene ice sheets as recently as 14–15,000 years ago (Wright, 1983, 1984, West, 1968), and their present flora and fauna are derived from subsequent colonization from the south.

Solem (1984), in a global review of snail diversity, has suggested that snail faunas of areas subject to such drastic changes may be far from the maximum diversity which the habitat could sustain. Evidence that niches are not always filled, nor constrained by competition comes also from studies on other communities and guilds (Lawton, 1984 and others in Strong, Simberloff, Abele & Thistle, 1984). Differences between faunas in such recent environments may depend on the accident of which species were available to colonize the newly available territory.

In this context, differences in the structure of the regions could also be important. The coastal zone of British Columbia is part of a narrow strip running from Alaska to California, bordered to the east by high mountain ranges, on and behind which climatic regimes are drastically different. Belts of arid and alpine zones seal off the coastal strip from the interior. Pleistocene movements of fauna and flora have been largely north-south, with a similar pattern of succession and regression in each interglacial or interstadial (Heusser & Heusser, 1981).

By contrast, oceanic climate influences penetrate far into the Eurasian continent (c.f. discussion in Cain, 1981). The east-west axis of European mountains may be responsible for a general impoverishment of the north European fauna and flora relative to that of the Appalachians, east of the American continental divide (e.g. MacArthur, 1972). Nevertheless, it is clear that the present snail fauna of north-west Europe derives from movements on an east-west as well as a north-south axis, and that the structure of the mountain ranges, especially of the Alps and Pyrenees has favoured speciation and local radiations (Kerney & Cameron, 1979). In north America, similar, indeed greater, local radiations have occurred in the more southerly Appalachians and Ozarks (Pilsbry, 1939-1948) but not to the west of the northern Cascades, where the coastal forest is hemmed in by alpine environments hostile to snails. The European snail fauna may have a greater diversity of origins than that of British Columbia: given the very short time (in evolutionary terms) that each area has been occupied by forest, such a difference would be more important than evolution *in situ* (Solem, 1984).

Western European forests are also more heterogeneous than those of British Columbia. In particular, submontane and hilly regions may have intimate mixtures of coniferous, mixed and broadleaved forests. Opportunities for species originating in one to colonize the other are great.

Other effects might delay the filling of niches. Many families of snails show considerable morphological conservatism (Cain, 1977, 1981). The markedly bimodal nature of the height/diameter scatter for pulmonate land snails indicates that, in most environments, there is an adaptive trough between the modes that is rarely crossed. The absence of large, tall-spired species in coastal British Columbia may be a consequence of a lack of appropriate ancestors in areas from which colonization took place. Even temperate, deciduous forests in North America lack large tall-spired species (Coney, Tarpley, Warden & Nagel, 1982; Cain, 1981).

Although the European faunas discussed here are more diverse than that of British Columbia, both taxonomically (Cameron, 1986) and in terms of size and shape, the latter nevertheless contains some forms (e.g. *Haplotrema*) not present in Europe. Whether this is another accident of history, or a consequence of the prior appropriation of the relevant niche by the great diversity of smaller, but similarly shaped Zonitids in Europe remains to be determined.

Given the still scanty knowledge of the determinants of land-snail niches, this discussion of causes of the differences between faunas is bound to be speculative. The obvious experiment, the introduction of species of sizes and shapes not represented in the indigenous fauna, is clearly objectionable on grounds of conservation.

For the same reason, detailed comparisons with faunas in very different climates would be premature. North-east Asian pulmonate faunas share with British Columbia (and the Pacific north-west generally) a deficiency in larger tall-spired shells. Cain (1981) points to the rigorous climatic regime there, in contrast to the milder climate at the same latitudes in Europe as a possible causal agent. This

explanation cannot, in itself, account for the British Columbian scatter.

The most diverse land-snail faunas known come from scrub woodlands on the North Island of New Zealand (Solem, Climo & Roscoe, 1981, Solem & Climo, 1985), where there is a fauna of more than 80 species, and where 60 species may be found in a single site. Analysis of these data reveals, however, that more than 80% of the species concerned are flattened or globular and have volumes less than 100 mm^3. Numbers of species in all other categories, and especially of high spired forms, are fewer than in European forests. Solem (1984) gives convincing reasons for the high level of taxonomic diversity in these New Zealand faunas (including a long history of climatic stability, enabling niches to be filled by both evolution and colonization). We now need to explore the reasons why this great diversity is expressed only in a very limited part of the spectrum of size and shape found elsewhere.

The incomplete convergence of the faunas compared here, and the results of Solem's (1984) survey suggest that, in certain circumstances, empty niches may occur in snail faunas. If further confirmed by other studies, this would in turn suggest that interspecific competition is by no means the most important determinant of snail fauna diversity, whether considered taxonomically or morphologically (Strong *et al.*, 1984). It does not follow that shell size and shape are of no adaptive significance. Cain (1977, 1981) shows that there is good reason to think that the mechanical consequences of modes of life exert powerful selective forces which constrain the range of morphologies actually found.

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APPENDIX

Lists of species found in the studies used in this paper. Asterisks mark species in genera common to both regions, and found in these studies.

(a) Species recorded in British Columbia, and their frequencies of occurrence. For details of sites etc. see text and Cameron (1986).

Allogona townsendiana	0.11	*Pristiloma lansingi*	1.00
**Carychium occidentale*	0.53	*Pristiloma stearnsi*	0.37
**Cionella lubrica*	0.16	**Punctum conspectum*	0.37
**Columella edentula*	0.95	**Punctum randolphi*	1.00
**Discus cronkhitei*	0.16	*Striatura pugetensis*	1.00
**Euconulus fulvus*	0.79	*Triodopsis germana*	0.63
Haplotrema sportella	0.89	**Vertigo andrusiana*	0.11
Haplotrema vancouverense	0.95	**Vertigo columbiana*	0.89
Microphysula cookei	0.16	**Vertigo rowelli*	0.11
Monadenia fidelis	0.84	*Vespericola columbiana*	0.89
**Nesovitrea binneyana*	1.00	**Vitrina alaskana*	0.11
Planogyra clappi	1.00	*Zonitoides arboreus*	0.26
Pristiloma johnsoni	0.11		

(b) Species recorded in 3 European studies, with frequencies of occurrence for those of Cameron (1973) and Körnig (1966). For details see text. Note that *Cionella* is given here as *Cochlicopa*.

	South Downs Cameron (1973)	German beechwoods (Körnig, 1966)	German conifer woods (Schmid, 1966)
Abida secale	0.08	0.10	—
Acanthinula aculeata	0.29	0.30	+
Acicula fusca	0.83	—	—
Acicula polita	—	—	+
Aegopinella nitens	—	0.65	+
Aegopinella nitidula	1.00	0.70	—
Aegopinella pura	0.92	0.95	+
Arianta arbustorum	0.12	0.05	—
Azeca goodalli	—	0.50	—
Bradybaena fruticum	—	0.40	—
Bulgarica cana	—	0.10	—
**Carychium tridentatum*	1.00	0.65	+
**Carychium minimum*	—	0.05	+
Cecilioides acicula	0.04	0.15	—
Cepaea hortensis	0.62	0.60	+
Cepaea nemoralis	0.54	0.75	+
Clausilia bidentata	0.71	0.90	—
Clausilia dubia	—	0.25	—
Clausilia parvula	—	0.15	—
**Cochlicopa lubrica*	0.51	0.40	+
**Cochlicopa lubricella*	0.20	—	—
Cochlodina laminata	1.00	1.00	+
**Columella edentula*	—	0.05	+
**Discus rotundatus*	1.00	1.00	+
Ena montana	—	0.85	+
Ena obscura	0.75	0.90	+
**Euconulus fulvus*	0.54	0.60	+
Euomphalia strigella	—	0.05	—
Helicigona lapicida	0.18	0.75	—
Helicodonta obvoluta	0.50	0.95	+
Helix aspersa	0.88	—	—
Helix pomatia	—	0.95	+

	South Downs Cameron (1973)	German beechwoods (Körnig, 1966)	German conifer woods (Schmid, 1966)
Iphigena pliculata	—	0.20	—
Iphigena ventricosa	—	0.55	—
Isognomostoma isognomostoma	—	0.45	—
Laciniaria biplicata	—	0.35	+
Macrogastra rolphii	0.75	—	—
Monacha cantiana	0.04	—	—
**Nesovitrea hammonis*	*	0.05	+
Orcula doliolum	—	0.25	—
Oxychilus alliarius	0.83	0.25	—
Oxychilus cellarius	0.79	0.90	+
Oxychilus helveticus	0.13	—	—
Perforatella incarnata	—	—	—
Pomatias elegans	1.00	—	—
**Punctum pygmaeum*	0.54	0.25	+
Semilimax semilimax	—	0.05	+
Trichia hispida	0.67	0.85	+
Trichia plebeia	—	0.05	—
Trichia striolata	0.62	—	—
**Vertigo pusilla*	—	—	+
**Vertigo substriata*	—	—	+
Vitrea contracta	0.96	0.80	—
Vitrea crystallina	0.04	0.10	+
Vitrea diaphana	—	0.40	—
**Vitrina pellucida*	0.58	0.55	+
Zenobiella subrufescens	*	—	—

*Species not recorded in the sites chosen for calculation of frequencies, see text.

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THE GENITALIC, ALLOZYMIC, AND CONCHOLOGICAL EVOLUTION OF THE EASTERN NORTH AMERICAN TRIODOPSINAE (GASTROPODA: PULMONATA: POLYGYRIDAE)

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ABSTRACT

The 40 species of triodopsines in eastern North America are useful for evolutionary studies because of their diverse genitalic and conchological radiations. Previous monographs were based on shells, many features of which are subject to convergence.

Dissection of the uneverted penial tubes revealed a morphological diversity that was classified into 10 characters comprising 60 character states. Cladistic analysis yielded a single most parsimonious tree with a consistency index of .970.

Starch-gel electrophoresis of foot tissue detected 74 alleles among 16 loci. Cladistic analysis using the independent alleles model resulted in a consensus, maximum-parsimony tree with a consistency index of .950. Electrophoresed populations were divided into two equal subsets for rooted distance-Wagner analyses based on Prevosti distances. The resulting trees had cophenetic correlations of .897 and .883.

The anatomical and allelic cladograms and the two genetic-distance trees were weighted according to the sizes and reliabilities of the data bases used in their construction. Branch-by-branch comparison of the four weighted trees produced a consensus phylogeny that was quite robust, and with only a few species remaining problematic due to incomplete or conflicting data.

Supraspecific revision based on this consensus phylogeny divides eastern triodopsines into four genera: *Neohelix* von Ihering, 1892; *Triodopsis* Rafinesque, 1819; *Webbhelix* Emberton, new genus; and *Xolotrema* (Rafinesque, 1819). The revision differs most strongly from previous classifications in its species groupings within the large genus *Triodopsis.*

Revision of the *Neohelix albolabris* group (the "white-lipped land snail"), based on 46 populations, discovered two new taxa: *N. solemi* and *N. albolabris bogani.* A cladogram (consistency index 1.00) based on genitalic morphometrics formed the basis for revision, which split the group into the *albolabris* and *alleni* groups. Shell differences among taxa are subtle and occasionally unreliable for identification, according to a multivariate discriminant analysis.

Genitalic and geographic comparisons between 25 pairs of sister taxa detected a pattern: sister taxa with virtually identical penial morphologies generally have peripatric geographic ranges, those slightly different are generally allopatric, those moderately different are sympatric, whereas those greatly different are parapatric. Population-level comparisons for 12 species failed to find any trace of reproductive character displacement. These results, as well as the pattern of genitalic convergences and the geographic stability of within-species genitalic morphology, led to the hypotheses that (1) peripheral isolates generally do not differentiate, (2) vicariant isolates generally differentiate slowly, (3) differentiation due to reproductive character displacement is moderate at most, and (4) major differentiation is rare, rapid, and occurs in isolates.

Shell evolution's pattern and inferred process differs among taxonomic levels. Genera show general conchological stasis despite extensive, overlapping ecological radiations; the process is probably canalization. Species groups show mosaic distributions of minor shell characters; the process is presumably genetic indeterminism of canalized developmental programs. Species and populations show two patterns: patchy, non-clinal variation in size and some aspects of shape and sculpture, probably induced by local microclimates; and iterated environmental correlations—e.g., between apertural obstruction and ground moisture, spire flatness and crevice-dwelling, and periostracal glossiness and water—presumably due to natural selection.

The nature and definition of a species in eastern triodopsines remains both a problem and a fruitful avenue of research. The many sympatric shell convergences between eastern triodopsines and the polygyrine genus *Mesodon* provide naturally replicated experiments in evolutionary morphology.

Key words: snails; evolution; genitalia; allozymes; shells; cladistics; character displacement; natural selection; convergence.

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INTRODUCTION

The Polygyridae are an autochthonous North American family of pulmonate land snails comprising approximately 260 species currently classified into 14 genera in 3 subfamilies (Pilsbry, 1940; Webb, 1954a; Hubricht, 1985; Richardson, 1986). This paper deals with eastern members of the subfamily Triodopsinae. Western triodopsines comprise the single genus *Vespericola* Pilsbry, 1939, which has some 9 species and ranges along the Pacific coastal zone from southern Alaska to northern California (Pilsbry, 1940; Roth, 1984) Eastern triodopsines, as revised in this paper, comprise the four genera *Neohelix* von Ihering, 1892, (7 species); *Triodopsis* Rafinesque, 1819 (26 species, not including the Siberian *"Triodopsis" supersonatum*—see Emberton, 1986); *Webbhelix* Emberton, new genus (1 species); and *Xolotrema* Rafinesque, 1819 (5 species). They range throughout temperate North America east of the Great Plains.

The eastern triodopsines are a common, large (8–40 mm), and sometimes dominant element of the leaf-litter invertebrate fauna. Eastern triodopsines are important for several reasons. (1) Because of their multiple sympatric conchological convergences on the polygyrine genus *Mesodon* (Pilsbry, 1940; Solem, 1976; Emberton, 1986), they contain superb naturally replicated experiments in evolutionary morphology (see Emberton, 1986). (2) Their diversity of complex penial morphologies (Webb, 1947–1980) makes them useful for testing the recent general hypotheses of Eberhardt (1985) concerning genitalic evolution. (3) Their substantial conchological variation (e.g., Vagvolgyi, 1968) makes them useful for advancing our very limited knowledge of the adaptive vs. ecologically induced components of shell shape in land snails (see review by Goodfriend, 1986). (4) The large size, high density, low vagility, and easy markability of many species make them useful subjects for generalizable studies in population biology (McCracken, 1976), population genetics (McCracken, 1980; McCracken & Brussard, 1980), life history and ecology (Vail, 1978; Emberton, 1981), and anatomy (Simpson, 1901; Emberton, 1985). (5) They are economically and ecologically important as the intermediate hosts of sometimes lethal parasites of elk, deer, and other

game and non-game mammals (e.g., Maze & Johnstone, 1986). (6) Their larger species are potentially of economic value as sources of anti-A agglutinin for typing human blood (Miles, 1983).

Previous monographic treatments of the eastern American triodopsines (Pilsbry, 1940; Vagvolgyi, 1968) were conchological.

The purposes of this paper are (1) to derive a robust phylogenetic hypothesis for the eastern triodopsines using two independent data sets: male genitalia and allozymes; (2) to revise the eastern triodopsines above the species level, based on this phylogeny; (3) to analyze phylogenetic patterns of variation in both genitalia and shell morphology and to generate hypotheses about the evolutionary processes which produced these patterns; and (4) to further revise the *Neohelix albolabris* group to the subspecies level.

The *Neohelix albolabris* group contains the largest, most conspicuous triodopsine snails. McCracken & Brussard's (1980) electrophoretic survey of "the white-lipped snail" (*Neohelix albolabris* [Say, 1816]) showed a confusing geographic diversity in this group which pointed out the need for taxonomic resolution using anatomical and conchological characters.

Penial morphology, presumed to be important in species recognition and of great potential value for the systematics of eastern triodopsines (Pilsbry, 1940; Webb, 1947–1980; Solem, 1976), has previously been exploited only to a very limited extent. There are three ways of studying penial sculpture in land pulmonates (Fig. 1): by killing and fixing the snail relaxed and extended from its shell, then dissecting open the uneverted penial tube (the dissective method); by killing and fixing the snail *in copulo* so as to keep its penis fully everted (the evertive method); or by clearing, staining, and mounting the uneverted penial tube (the slide-mount nethod). Until the beginning of Webb's publications in 1947, the only triodopsine species for which details of penial sculpture were known was *Neohelix albolabris,* illustrated by Binney (1851), Pilsbry (1894, 1940), and Simpson (1901); in all four of these papers it was studied by the dissective method. Webb (1947, 1948, 1952, 1954, 1959) studied 12 species and Grimm (1975) studied one species of eastern triodopsines by the evertive method and illustrated the general aspects of penial sculpture. Solem (1976) illustrated the dissected uneverted penial tubes of three species, thereby redemonstrating the efficacy of the dissective method and showing the wealth of sculptural detail omitted by Webb and Grimm.

The dissective method is in many respects superior to both the evertive and slide-mount methods (Fig. 1). Waiting for penial eversion, then killing and fixing without distorting the soft tissues, is labor-intensive and yields little additional information (but see Character 10 below). Clearing and mounting the uneverted penial tube is more time-consuming than cutting it open, and is much less effective for interpreting complex sculpture because of three-dimensional overlap further distorted by viewing through other tissues.

Thus the most obvious source of useful characters for phylogenetic analysis was penial sculpture as viewed by the dissective method. For this character set, 27 of the 40 species of eastern triodopsines had never been examined before, and, of those that had, only 3 had been illustrated in sufficient detail.

The other character set chosen for phylogenetic analysis was that of allozymes as viewed by horizontal starch-gel electrophoresis. Regardless of whether allozymes are adaptively significant (e.g., Hochachka & Somero, 1984; Nevo & Bar, 1976; Nevo et al., 1981; Nevo et al., 1982) or adaptively neutral (e.g., Kimura, 1979, 1982), they offer a morphological data set virtually independent of penial morphology. Four species of eastern triodopsines had previously been electrophoresed (McCracken & Brussard, 1980, as reevaluated by Emberton, McCracken & Wooden, in preparation). These were examined at 8 variable loci that showed sufficient variation to bode success for applying electrophoresis to the systematics of the entire group. Certain alleles of some loci had also been shown to be genetically heritable (McCracken, 1976, 1980).

The value of allozymes for systematic studies is well established (e.g., Avise, 1975; Sarich, 1977; Throckmorton, 1978; Davis, 1978; Nei et al., 1983; Patton & Avise, 1983; Buth, 1984). Although land-snail allozymes have been used extensively for studies on population genetics and breeding systems (reviewed by Clarke, 1978; Selander & Ochman, 1983; Selander & Whittam, 1983), only twice before have they been used for extensive phylogenetic studies. In neither of these previous efforts—on West Indian *Cerion* by Gould et al. (1975), and on Moorean *Partula*

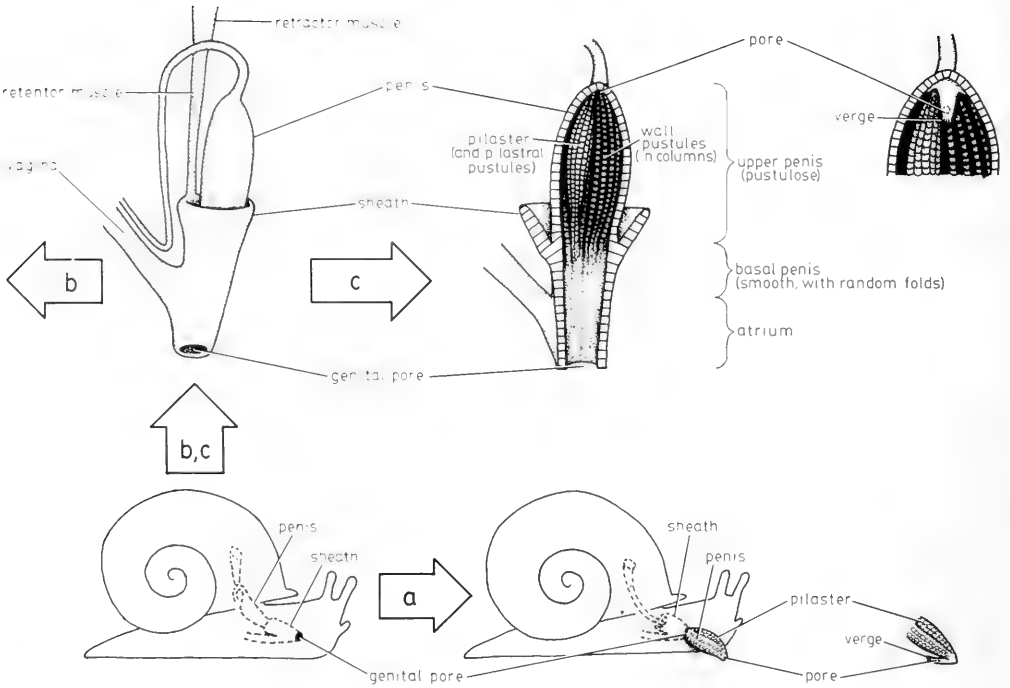

FIG. 1. Penial morphology of east American triodopsines: its major features and the three alternative methods for studying the sculpture of its functional surface. **a.** The evertive method. **b.** The slide-mount method (clearing and staining). **c.** The dissective method.

by Johnson et al. (1977)—was sufficient electrophoretic variation found to be of much value in reconstructing species-level phylogenies. Both these groups, however, appear to be relatively recent radiations (Woodruff & Gould, 1978; Murray & Clarke, 1980), much younger than eastern triodopsines (see Emberton, 1986).

Thus penial morphology and allozymes were chosen because of their independence from each other and because each promised to be rich in phylogenetically useful variation. To avoid circularity in evaluating conchological evolution, no shell characters were used for phylogenetic analysis.

Time constraints prohibited the use of other morphological character sets that previous studies had indicated were less information-rich than penial morphology and allozymes. Concerning radulae, Solem's (1976) study of three triodopsines (two of them sympatric) had found an "essential similarity", with, "in terms of basic structure and pattern of functioning, . . . no major differences between species, much less between genera". Likewise, Binney's (1878) sketches of the radulae of 9 other triodopsine species, although rather inadequate in detail, showed a similar lack of variation. The macro- and microstructure of the jaw promised little useful information, because Solem (1976) found "no significant differences" among three species of eastern triodopsines.

The size and shape of the hermaphroditic duct, talon, albumen gland, prostate, uterus, and spermatheca undergo such significant and extreme seasonal variation in one species of *Triodopsis* (Emberton, 1985) that the use of these characters for systematics would have had to have been cautious and labor-intensive. Likewise, considerable variations in the diameter and length of the ovotesticular lobes, the basal penis, the free oviduct, and the vagina correlate with changes in reproductive state (Emberton, 1985: figs. 5–7).

Preliminary studies (Emberton, unpublished data) showed that the internal structure of the functional vagina (the spermathecal or gametolytic duct) was identical in several triodopsine species. This lack of variation extended to several pairs of microsympatric species with similar shell sizes and penial morphology. The structure of the triodopsine functional vagina has been illustrated by

Binney (1851: pl. 7, fig. 4; pl. 8, fig. 3), Simpson (1901: pl. 8, fig. 11), and Grimm (1975: fig. 3A).

Technology for viewing the chromosomal bands of land snails (e.g., Babrakzai & Miller, 1975, 1984) seemed in too early a stage of development for a project of this scope. Simple chromosomal counts promised little insight, because an early study (Husted & Burch, 1947) of 17 species of polygyrids, including 6 triodopsines, found a diploid number of 58 in all except what was identified as *Triodopsis fraudulenta,* populations of which were reported to vary in diploid number from 58 to 62. Furthermore, the phylogenetic interpretation of chromosomal numbers can be highly problematic (e.g., Solem, 1978).

Thus this phylogenetic analysis of the eastern American triodopsines was restricted to male-genitalic and allozymic characters.

MATERIALS AND METHODS

Taxa studied

Neohelix von Ihering, 1892
- *albolabris* (Say, 1816)
- *alleni* (Sampson, 1883)
- *dentifera* (Binney, 1837)
- *divesta* (Gould, 1848)
- *lioderma* (Pilsbry, 1902)
- *major* (Binney, 1837)
- *solemi* Emberton, new species

Triodopsis Rafinesque, 1819
- *alabamensis* (Pilsbry, 1902)
- *anteridon* (Pilsbry, 1940)
- *burchi* Hubricht, 1950
- claibornensis Lutz, 1950
- *complanata* (Pilsbry, 1898)
- *cragini* Call, 1886
- *discoidea* (Pilsbry, 1904)
- *fallax* (Say, 1825)
- *fraudulenta* (Pilsbry, 1894)
- *fulciden* Hubricht, 1952
- *henriettae* (Mazÿck, 1877)
- *hopetonensis* (Shuttleworth, 1852)
- *juxtidens* (Pilsbry, 1894)
- *messana* Hubricht, 1952
- *neglecta* (Pilsbry, 1899)
- *obsoleta* (Pilsbry, 1894)
- *palustris* Hubricht, 1958
- *pendula* Hubricht, 1952
- *picea* Hubricht, 1958
- *platysayoides* (Brooks, 1933)
- *rugosa* Brooks & MacMillan, 1940
- *soelneri* (Henderson, 1907)
- *tennesseensis* (Walker & Pilsbry, 1902)
- *tridentata* (Say, 1816)
- *vannostrandi* (Bland, 1875)
- vulgata (Pilsbry, 1940)
- *vultuosa* (Gould, 1848)

Webbhelix Emberton, new genus
- multilineata (Say, 1821)

Xolotrema Rafinesque, 1819
- *caroliniensis* (Lea, 1834)
- *denotata* (Férussac, 1821)
- *fosteri* (F. C. Baker, 1932)
- *obstricta* (Say, 1821)
- *occidentalis* (Pilsbry & Ferriss, 1907)

Collections

Principal field work was conducted April-June 1982 in the eastern United States ("GS" series), and was supplemented by collections from southeastern Ohio in March-July 1979 ("Ohio" series), from the lower Ohio River Valley in April 1980 ("H" series), and from the southern Appalachian area in March-June 1983 ("SC" series). All collections were donated to the Field Museum of Natural History, Chicago. County-level localities, field numbers, and catalog numbers of dissected and electrophoresed material are listed under each species in the systematic reviews in Appendices B and C. Detailed locality data are available from the author on request or from the Field Museum catalog. Snails in each lot were individually marked on their shells: 1, 2, 3, etc. for snails from which tissue samples were taken; and A, B, C, etc. for snails that were not tissue-sampled. Appendices B and C record which individual snails from each lot were dissected, electrophoresed, and illustrated anatomically and/or conchologically.

Additional anatomical material (total 18 lots) was borrowed from the Field Museum (FMNH), the Academy of Natural Sciences of Philadelphia (ANSP), and the private collection of Mr. Leslie Hubricht.

For the *Neohelix albolabris* and *alleni* groups, 41 populations were collected or borrowed, and 5 additional populations were studied from published anatomical illustrations.

Dissections

The uneverted penial tubes of 252 snails from 108 populations comprising all 40 of Hubricht's (1985) species were dissected. Most populations were collected in the early

spring, but to make a crude check for significant seasonal variation which might bias interspecific comparisons, three *T. tridentata* from Strouds Run State Park, Ohio, were dissected, each at a different stage in the life cycle of this species: mating-ready neoadult (FMNH 209209, specimen C); post-mating neoadult (FMNH 209536, specimen C); and overwintered, mating-ready, old adult (FMNH 209209, specimen D) (see Emberton, 1985).

Whenever possible, at least three adults of each species were dissected. Because of the limitations of available material, however, 10 species were represented by only two dissections each *(X. obstricta, X. caroliniensis, T. picea, T. claibornensis, T. fraudulenta, T. rugosa, T. vultuosa, T. cragini, T. alabamensis,* and *T. neglecta)*, and 5 species were represented by only a single dissection each *(X. occidentalis, T. henriettae, T. discoidea, T. fulciden,* and *T. pendula).* The remaining 25 species were represented by three or more dissections each, usually with at least three from a single population.

A representative dissection was illustrated for 39 of the 40 species, by means of a drawing tube attached to a Zeiss dissecting microscope. Relaxed specimens were used for 35 species, but because of limited material *X. occidentalis, T. complanata, T. obsoleta,* and *T. fallax* were represented by contracted specimens. *T. rugosa* became available too late to be illustrated.

Comparative anatomies of eastern American triodopsine outgroups were available in published illustrations. According to Emberton's (1986) phylogenetic analysis of the Polygyridae, eastern triodopsines are the most primitive group in the family, and their closest outgroups are the western American triodopsine *Vespericola,* and the ashmunellines *Cryptomastix* (western) and *Allogona* (western, with one eastern species: *A. profunda*). Penial-morphological data on these genera were available from Pilsbry (1940) and Webb (1948, 1968, 1970a, 1970b, 1970c). A more distant polygyrid outgroup of the triodopsines is the ashmunelline genus *Ashmunella,* the penial morphology of which was gotten from Pilsbry (1940) and Webb (1954). The closest non-polygyrid outgroups of triodopsines, according to the Emberton (1986) hypothesis, are the Corillidae, Ammonitellidae, and Oreohelicidae. In the Corillidae, only the external, uneverted penial morphology of one species is known (Solem, 1966); in the Ammonitellidae, limited details of the penial sculpture are known for *Polygyrella, Polygyroidea,* and *Ammonitella* (Pilsbry, 1939: figs. 369 #5a, 371 #5a, 373 #1g); in the Oreohelicidae, penial sculpture is known for a number of *Oreohelix* species (Pilsbry, 1939; Solem, 1978b). Another, more distant outgroup to the triodopsines which was considered were the Camaenidae, the penial anatomy of many species of which is known through the work of Wurtz (1955) and Solem (1979, 1981a, 1981b, 1984). See Tillier (1986) for an alternative view on triodopsine outgroups.

Additional methods were used for the study of the *Neohelix albolabris* and *alleni* groups. In order to quantify genitalic differences among taxa, 7 measurements were taken from one dissection per population for three populations each of *N. alleni* (pooling the two subspecies, which did not differ in the measurements taken—see Fig. 3), *N. albolabris albolabris, N. albolabris bogani* Emberton, new subspecies, *N. major,* and *N. solemi.* For these measurements, the most relaxed specimens were chosen from widely distributed populations. The measurements were: (1) the length of the penis, in mm, from its junction with the vagina to the internal apex of the dissection; (2) the number of pilastral lappets (this and other terminology is defined later) per 2.6 mm at the midpoint of the pilaster; (3) the number of columns of wall pustules per 1.3 mm, measuring transversely across the penial wall adjacent to the pilaster about two-thirds of its distance from the internal penial apex; (4) the length of the verge in mm; (5) the maximum width of the pilaster in mm; (6) the distance in mm from the external apex of the penis to the midpoint of the origin of the penial retractor muscle on the vas deferens; and (7) the length of the vas deferens, in mm, from where it bends at the external junction of the penis and vagina to its point of insertion at the external penial apex.

Shell analysis

Phylogenetic analysis to the species-group level was entirely free of consideration of shell morphology. In the systematic reviews, however (Appendices B and C), comparative conchological descriptions are included to allow identification to species group from shells alone. To aid identification, a representative shell for each of 39 species (all but *T. rugosa*) was illustrated in two views: perpendicular to the plane of the aperture, and in the

plane of the aperture while parallel to the axis of rotation. These views were chosen because they simultaneously show as many important shell features as possible, including apertual dentition, apertural dishing, apertural lip thickness, pre-apertural deflection of the body whorl, umbilicus, height, surface striae, and, in a rough way, whorl count. The shell drawings were made using a drawing tube mounted on a Zeiss dissecting microscope. For most species, the illustrated shell was from the same population from which the penial morphology was illustrated.

Shells of the *Neohelix albolabris* and *alleni* groups were studied in much greater detail. Despite a great similarity in the overall aspect of the shells, and an overlap in shell size among the 6 species and subspecies of this group, subtle conchological differences were apparent. In order to quantify these differences and to objectively test their reliability for identifying the taxa, a multivariate discriminant analysis was performed, beginning with a set of 11 measurements on 55 shells from 28 populations. These populations, their species or subspecies, and the identification numbers of the shells measured from each, are listed in the first three columns of Table 6. For each of the six taxa, a set of populations was chosen which appeared to include its full range of shell variation; from each population, all undamaged adult shells were measured if there were no more than three—if there were more than that, the three shells showing extremes in the population's variation were chosen for measurement.

There were 8 shell variables in which the 6 species and subspecies of the *N. albolabris* and *N. alleni* groups appeared to differ: relative spire height (henceforth called RELSPIRE), whorl expansion rate (WHRLEXPN), relative width of the apertural lip (RELLIP), relative size of the baso-columellar lip node (RELNODE), relative degree of pre-apertural deflection of the body whorl (RELDEFL), density of surface striae (STRIAE), color (BROWN), and sheen (GLOSSY). These variables and the method for quantifying each are listed in Table 5. STRIAE was a direct count, BROWN and GLOSSY were rank measurements, and the remaining 5 (RELSPIRE, WHRLEXPN, RELNODE, RELLIP, AND RELDIFL) were ratios of directly measured or calculated distances. The 11 measurements from which the 8 variables were derived are listed as column headings in Table 6.

Electrophoresis

Posterior foot tissues ("snail tails") were excised from field-activated snails and stored in cryogenic vials in liquid nitrogen. Horizontal starch-gel electrophoresis followed methods of Selander *et al.* (1971) and Shaw & Prasad (1970), as modified by Davis et al. (1981). Twelve enzyme systems yielding 16 loci were used: Sordh, Mdh-1 & 2, Me, Icd, Pgd, Gd-1 & 2, Sod-1 & 2, Got-1 & 2, Pgm, Lap, Mpi, and Gpi (see Appendix A). These loci were chosen because they were genetically interpretable, because they represent a diversity of metabolic pathways, and because several of them have a proven heritability (McCracken, 1976, 1980). Deliberately excluded were enzymes that have been shown to be environmentally induced in pulmonates: esterases (Oxford, 1973, 1978), lactate dehydrogenase (Gill, 1978a), acid phosphatase, and alpha-glycerophosphate dehydrogenase (Gill, 1978b). Complete electrophoretic procedures are given in Appendix A.

The electrophoresed material comprised 249 snails from 64 populations representing 35 of the 40 Hubrichtian (1985) species of eastern triodopsines. The 5 species for which tissue samples were lacking were *T. discoidea, T. fallax, T. obsoleta, T. rugosa,* and *T. soelneri.* Three electrophoresed species had incomplete data: *X. fosteri* (missing Gd-1, Gd-2, and Sod-2), *T. fulciden,* and *T. henriettae* (both missing Gd-1 and Gd-2). All other species (32 total) were represented by at least one population with complete data for all 16 loci.

Seventeen species were represented by a single electrophoresed population each *(T. albamensis, T. burchi, X. caroliniensis, T. claibornensis, T. complanata, X. fosteri, T. fraudulenta, T. fulciden, T. henriettae, N. lioderma, T. messana, T. neglecta, T. pendula, T. picea, T. platysayoides, N. solemi* and *T. vannostrandi);* 13 species were represented by two populations each (*T. anteridon, T. cragini, X. denotata, N. dentifera, N. divesta, T. hopetonensis, T. juxtidens, W. multilineata, X. occidentalis, T. palustris, T. tennesseensis,* and *T. vulgata;* four species were represented by three populations each (*N. albolabris, N. major, X. obstricta,* and *T. vultuosa*); one species was represented by 5 populations (*N. alleni*); and one species was represented by 6 populations *(T. tridentata).* Catalogue numbers of the voucher specimens for all electrophoresed populations are given in col-

umn 2 of Table 2, and in Appendices B and C. Of the total 64 populations, 50 had complete electrophoretic data and 14 had missing data for one to 7 loci.

The number of snails electrophoresed per population (Table 2, column 3) ranged from one to 12, with a mean of 3.9 and a standard deviation of 2.5.

The closest outgroup of eastern triodopsines from which comparative material was available was *Allogona profunda* (Say, 1821), of which two populations with sample sizes of 2 and 10 were electrophoresed.

Data analysis

Penial morphology was analyzed cladistically (Hennig, 1966; Eldredge & Cracraft, 1980; Wiley, 1981). A character-state phylogeny was proposed for each character, using criteria reviewed by Emberton (1986), and its polarity was determined by outgroup comparison (e.g., Watrous & Wheeler, 1981). A taxon-by-character-state matrix was prepared using additive binary coding (Farris *et al.,* 1970). Cladograms were generated from this matrix using the Wagner criterion of unrestricted parsimony (Kluge & Farris, 1969; Farris, 1970), using global branch swapping to approach heuristically the most parsimonious set of trees. The PAUP program (Swofford, 1983) was used for computing the trees. These trees were visually compared branch-by-branch, and each discrepancy was resolved based on which combination of convergences and reversals seemed biologically most plausible. The final result of these comparisons was a single, most parsimonious cladogram that was designated the "Anatomy Tree".

Electrophoretic data were analyzed both cladistically and phenetically. Cladistic analysis employed the independent alleles model (Mickevich & Johnson, 1976), by which alleles not present in the outgroup are considered apomorphous. *Mesodon* was used as the outgroup, because it was the only other polygyrid group for which a comparable electrophoretic data set was available (Emberton, 1986). For each eastern-American triodopsine species, the presence or absence of each apomorphous allele was binary-coded. The resulting data matrix was analyzed by PAUP (Swofford, 1983), using global branch swapping to obtain the first 50 trees with equal, maximum parsimony. These trees were then compared branch-by-branch to determine the most frequently occurring configuration of each branch. In this manner, a single maximum-parsimony, consensus cladogram was arrived at, and was designated the "Alleles Tree."

For phenetic treatment, the electrophoretic data set was divided into two subsets, the first consisting of 32 species plus the outgroup *(Allogona profunda),* each represented by a single population with complete data for all 16 loci. The second subset consisted of three species not included in the first subset, plus additional populations of 18 species in the first subset, plus two outgroups (*A. profunda and Mesodon zaletus* [Binney, 1837]), for a total of 33 populations. In this second subset, all loci with incomplete data were deleted, leaving 8 loci: Sordh, Mdh-1, Mdh-2, Pgd, Sod-1, Got-1, Pgm, and Gpi. For each of the two subsets, Prevosti distances (Wright, 1978) among populations were calculated and subjected to the distance-Wagner procedure (Farris, 1970), with branch-length optimization, using NT-SYS computer programs (Rohlf et al., 1972). The resulting trees were designated the "Wagner-1 Tree" and the "Wagner-2 Tree."

The Anatomy, Alleles, Wagner-1, and Wagner-2 Trees were combined to produce a Consensus Tree in the following manner. Each of the four trees was weighted by a combination of two criteria: the number of data units and the relative reliability of the data units. The data units were considered to be character-state transformations in the Anatomy and the Alleles Trees, and to be alleles in the Wagner-1 and Wagner-2 Trees. The reliability of anatomical data-units relative to allozymic data-units was estimated by dividing the number of convergences and reversals in the Anatomy Tree by the number of convergences and reversals in the Alleles Tree. Multiplying this reliability index times the number of anatomical character-state transformations gave a relative weight for the Anatomy Tree. The relative weight of the Alleles Tree was taken as the number of single-allelic transformations. Relative weights of the Wagner-1 and Wagner-2 Trees were considered to be to the number of alleles comprising the data subset from which each tree was calculated. Using these weightings to resolve conflicts, the four trees were visually compared branch-by-branch to arrive at a Consensus Tree.

For a more detailed cladistic analysis of the *Neohelix albolabris* and *alleni* groups, addi-

tional anatomical character-state transformations were proposed based on the quantitative comparisons in penial morphology. All the available transformations were then used to construct a maximum-parsimony cladogram by hand.

Multivariate discriminant analysis of shells of the *Neohelix albolabris* and *alleni* groups employed SAS software (SAS Institute, 1982). The 6 taxa were discriminated on the basis of 8 shell variables (Table 5). Eight of the 55 measured shells had an incompletely matured apertural lip (Table 6, last column), which affected the values of RELNODE and RELLIP, therefore these shells were deleted from the analysis.

Patterns of genitalic evolution

Patterns of evolution in penial morphology were analyzed by comparing sister taxa (species or species clusters appearing dichotomously in the Consensus Tree). For each of 25 sister taxa, the difference in penial morphology was ranked as great, moderate, slight, or none; and the geographical relationship of their ranges was classified as allopatric, sympatric, parapatric, or peripatric (in which one taxon is a small-ranged endemic peripheral to the much broader range of the other). Geographic ranges were gotten from Hubricht (1985).

The importance of reproductive character displacement was assessed by comparing, for each of 12 species, populations sympatric vs. allopatric with another triodopsine species of similar shell size and shape. Table 9 lists the species, the sympatric species, the localities of compared populations, and the number of dissections per population. Allopatric populations of *T. tridentata* were compared with populations sympatric with *T. vulgata, X. obstricta, T. picea,* and *T. juxtidens;* likewise *T. vulgata* was tested for penial differences due to sympatry with *X. denotata, T. tennesseensis,* and *T. tridentata.* Also tested were *N. albolabris* against *N. alleni* and *N. dentifera; T. juxtidens* against *T. tridentata;* and both *N. alleni* and *N. dentifera* were tested against *N. albolabris.*

Patterns of shell evolution

To analyze conchological evolution at the generic and species-group levels, a representative shell was chosen for each species and was mounted in its proper position on the Consensus Tree. Patterns of change through time were interpreted under the assumptions that (1) the Consensus Tree was an accurate estimate of true phylogeny, and (2) the shell morphology of each (unknown) ancestor was between the morphologies of its extant descendents.

Patterns at the species level were assessed using Vagvolgyi's (1968) conchological monograph, in which the ranges of basic shell measurements and ratios are listed within each species description. Vagvolgyi's total data base comprises 31,269 shells from 556 museum lots. For the present analysis, his data were compiled into tabular form, and a "diameter range" index (Solem, 1981a) was calculated for each species: the greatest minus the least measured shell diameter, divided by the least, and expressed as a percent.

TAXONOMIC HISTORY

Triodopsis and *Xolotrema* were erected by Rafinesque in 1819 to separate *tridentata, denotata,* and other tridentate North American species from *Helix,* then a massive genus comprising most of the world's land snails. The new generic names were largely ignored, however (all polygyrids going by the name *Polygyra* Say, 1818), until "Tryon (1867), Binney & Bland (1869), and later authors, following von Martens (1860) used *Triodopsis* for all of the depressed, two- or three-toothed [land shells] of the eastern United States, and *Mesodon* for the more capacious, subglobose species with a small parietal tooth, or toothless [and thus abandoned the name *Xolotrema*]" (Pilsbry, 1940). Many authors (e.g., Simpson, 1901; F. C. Baker, 1939) continued, however, to synonyomize *Triodopsis* and *Mesodon* under the blanket genus *Polygyra.* It wasn't until Pilsbry's (1940) monograph on North American land snails that *Triodopsis* was clearly characterized anatomically, was distinguished anatomically from *Mesodon,* and was recognized as covering most of the wide range of shell shapes also covered by *Mesodon* but formerly erroneously divided between the two genera.

In this monograph, Pilsbry (1940) divided *Triodopsis* into the four subgenera *Triodopsis s. str.* Rafinesque, 1819; *Cryptomastix* Pilsbry, 1939; *Xolotrema* Rafinesque, 1819 (bringing this name back from obscurity); and

Neohelix von Ihering, 1892, based on shell shape and reproductive anatomy, with *Cryptomastix* disjunct in the Pacific Northwest. Pilsbry's taxonomy of eastern *Triodopsis* (i.e., the eastern triodopsines) was almost exclusively based on shell morphology, despite the fact that he illustrated the reproductive systems of several species. He recommended that future revisions make use of penial morphology.

Additional species and subspecies of *Triodopsis* were subsequently described by Lutz (1950) and Hubricht (1950, 1952, 1958). A summary of new and emended taxa from 1948 to 1984 was provided by Miller *et al.* (1984).

Webb (1947a, 1947b, 1948, 1952, 1954, 1959) published a series of reports on the reproductive behavior and anatomy of selected species of triodopsines, and pointed out—as Pilsbry had predicted—important variation in penial sculpture, upon which he based several taxonomic changes. In his 1952 paper, Webb elevated *Xolotrema* to a full genus (defined as possessing a penial verge) and transferred the subgenus *Neohelix* to it. In 1954, Webb elevated the Pacific Northwestern subgenus *Cryptomastix* to generic level within the new subfamily *Ashmunellinae,* thereby restricting *Triodopsis* to eastern North America. Also based on penial morphology, Webb erected the subgenus *Wilcoxorbis* for *Xolotrema fosteri* (Webb, 1952), the subgenus *Haroldorbis* for *Triodopsis cragini,* and the section *Shelfordorbis* for *Triodopsis vulgata* (Webb, 1959).

Vagvolgyi's (1968) monograph, "Systematics and Evolution of the Genus *Triodopsis* (Mollusca: Pulmonata: Polygyridae)", summarized a massive amount of new data on conchological variation. This revision was based solely on shells and ignored Webb's (1947–1961) anatomical work and validly proposed supraspecific taxa (see Grimm, 1975; Solem, 1976). Additional shortcomings of this work were that (1) the numerical formulae used for separating and defining taxa were arbitrary, based on neither multivariate nor any other objective criterion; (2) designation of "hybrids" was based on the untested criterion of high within-populational variation, the presence of which can have other explanations; and (3) the ecological descriptions were often arrived at by comparing species ranges with broad-scale vegetation maps, thereby sometimes missing important finer-grained ecological differences (L. Hubricht, personal communication; personal observations).

Vagvolgyi's *Triodopsis copei* (Wetherby) was subsequently split into the three species *cragini, vultuosa,* and *henriettae* by Cheatum & Fullington (1971) in their monograph of Texas polygyrids.

Grimm (1975) gave brief comparative descriptions of *Triodopsis* and its species groups, and summarized his systematic conclusions concerning the *T. fallax* group based on a ten-year study of geographic shell variation, laboratory hybridization, and, to lesser extent, penial morphology. Grimm's conclusions based on these (largely undocumented) studies were concordant with those earlier postulated from geographic shell variation by Hubricht (1953), but ran counter to those of Vagvolgyi (1968).

Solem's (1976) "Comments on Eastern North American Polygyridae" compared the sympatric, conchologically similar *Neohelix divesta* and *N. albolabris* with each other and with three sympatric, conchologically similar species of *Mesodon* in shell, radular structure, jaw structure, external aspect of the reproductive system, and dissected penial morphology. Comparative data on the rare *Triodopsis platysayoides* were also included. Solem emphasized the need for sympatric-species comparisons to establish criteria for distinguishing allopatric species, and showed through adequate illustrations that penial morphology was an even richer source of systematically useful characters than Webb's illustrations had indicated.

McCracken & Brussard's (1980) study of electrophoretic variation among populations of the "white-lipped land snail", although incorrect in many of its conclusions due to taxonomic errors (Emberton, McCracken, & Wooden, in preparation), showed the presence of significant allozymic variation within *Neohelix* and demonstrated the heritability of several loci in a New York population of *N. albolabris.*

Emberton (1985) dissected a temporal series of *Triodopsis tridentata* and found that extreme seasonal variation ruled out reproductive-organ volume and, to some extent, organ length as useful systematic characters for triodopsines.

Hubricht's (1985) book of range maps and ecological sketches of eastern North American land snails discarded most of Vagvolgyi's (1968) species-level taxonomic changes and elevated several of Pilsbry's (1940) subspe-

cies and the one subspecies of Lutz (1950) to full species, resulting in 40 total species; taxonomy between the genus and species levels was not included. Richardson's (1986) bibliographic catalog of polygyrid species did not incoprorate Hubricht's (1985) changes.

GENITALIC ANALYSIS

Variation

The eastern-triodopsine penis and its major structural features are presented diagrammatically in Fig. 1. The uneverted penis is held internally by a single retractor muscle attached to the vas deferens near the penial apex. The penial tube varies from short to extremely long, from cylindrical to clubbed. The position of entry of the vas deferens at the ejaculatory pore varies from terminal to subterminal. The collar-like muscular sheath, which circularly attaches to the basal penis and connects to the vas deferens via the retentor muscle, varies from short (covering only the basal fourth of the penis) to very long (covering the entire penis).

Dissection of the uneverted penial tube reveals its ornately sculpted functional surface (Fig. 1). The dorsal pilaster is a longitudinal outgrowth of the penial wall; it varies among species in both length and surface sculpture. The penial wall (exclusive of the dorsal pilaster) is covered with rows of pustules. These pustules vary in size and shape among species, and are sometimes lacking. The pustular rows vary in pattern; when their pustules are absent they appear as low, smooth ridges. The area surrounding the ejaculatory pore may be flat or may be elongated as a flap-like, conical verge of variable size and shape. Other features which also may be present (but are not shown in Fig. 1) are a smooth ventral sperm groove; a fleshy, knob-like peduncle beneath the ejaculatory pore; and a low ventral pilaster.

Proximal to the upper, sculpted region of the penis lies the basal penis. This region is smooth, lacking pustules. Its walls vary from thin with random folds, to thick and muscular with regular folds produced by both longitudinal and circular muscle bands. Proximal to the basal penis, between the vaginal opening and the genital pore, is the atrium. The wall of this region is always smooth and thin, bearing random folds.

Variation within any given population was minor in sculptural details, but major in such elastic features as penis length, sheath length, retractor-muscle and retentor-muscle lengths, and the configuration of folds in the basal penis. Much of this variation seemed to correlate with the contractile condition of the specimen.

Seasonal variation in penial morphology in the studied population of *Triodopsis tridentata* was slight. The post-mating snail had a thinner wall, and its pustules were somewhat thin and flap-like compared to the more prominent, robust pustules of both mating-ready snails. The distribution and relative sizes of the pustules, however, remained constant.

In all 13 species for which more than one population was dissected, upper penial sculpture was remarkably uniform. An example of this geographic stability is illustrated in Fig. 3, which shows the penial morphologies of two populations of *Neohelix alleni* separated by the Mississippi River Valley. Judging both from the wide range disjunction of this species (Fig. 49) and from the fossil-palynological evidence concerning its deciduous-forest habitat (Delcourt & Delcourt, 1981), these two populations had been genetically isolated for at least 20,000 years, which is probably equivalent to at least half as many generations (see McCracken, 1976). Nevertheless, these populations had accumulated only minor differences in penial sculpture: the eastern population *(N. alleni fuscolabris)* differed from the western *(N. alleni alleni)* only in its somewhat larger verge and in having its pustulose region descend approximately 20% lower.

Because of this general morphological conservatism, the penial morphology of each species could be adequately represented by a single illustration (Figs. 2–18).The only species not illustrated *(Triodopsis rugosa)* was very similar to *T. fulciden* (Fig. 18b).

Descriptions

Measurements in the following descriptions were taken solely from the illustrations (Figs. 2–18) and do not in any way reflect natural variation. Penis length was measured from the apex to the genital pore. The verge was measured from its dorsal side. The terms "large", "small", etc. are used relative to total penis length.

Neohelix albolabris (Say, 1816)—Dissections: 27 from 14 populations. Fig. 2d-g. Length 17 mm. Shape cylindrical, the apical

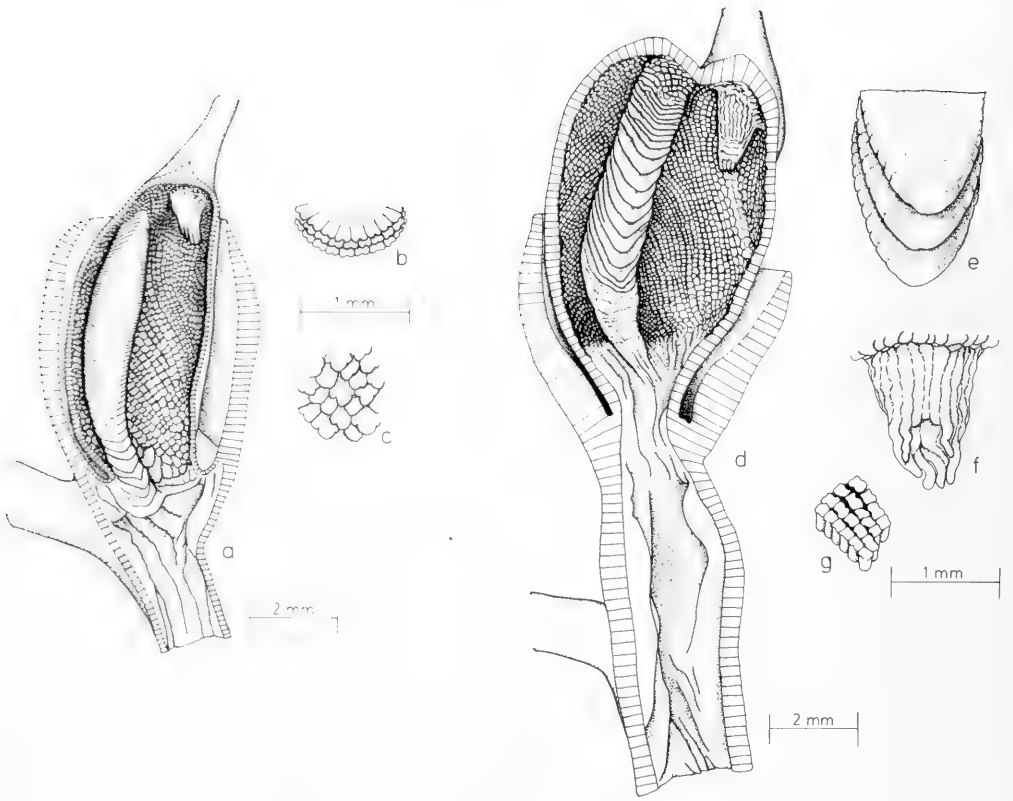

FIG. 2. Opened uneverted penial tubes. **a.** *Neohelix dentifera* (Binney, 1837). FMNH 214810 #8 (also dissected #1, 4; FMNH 214809 [sympatric with *Neohelix albolabris*] #2, 7, 14). **b.** Diagrammatic detail of 3 lappets from center of pilaster of a, showing substructure of pustules. **c.** Diagrammatic detail of central wall pustules of a, showing lateral cusps. **d.** *Neohelix albolabris* (Say, 1816). FMNH 214920 (sympatric with *Neohelix dentifera*) #14 (also dissected #9, 11, 17; and 8 other populations——see Appendix B). **e.** Detail of 3 lappets from center of pilaster of d, showing substructure of apparently fused pustules. **f.** Detail of other side of verge in d, showing opening of vas deferens. **g.** Detail of wall pustules of d.

half enlarged. Ejaculatory pore terminal, on a verge. Verge large (length 1.5 mm), terminal, dorso-laterally compressed, back-pointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 6 terminal papillae (Fig. 2f). Dorsal pilaster long (7 mm) and broad (mid-width 1.3 mm), and superficially resembling a stack of tongue-like lappets with edges slightly convex and regularly marked with slight indentations (Fig. 2e). Basal half of the penis smooth with random folds; upper half uniformly sculpted with 25–35 adjacent, generally unmerging, equilateral columns of distinct, equal-sized pustules (Fig. 2g), radiating from the pore region. Sheath enclosing less than half of the upper, sculpted region of the penis.

Neohelix alleni (Sampson, 1883)—Dissections: 15 from 8 populations. Fig. 3. Length 20 mm. Shape cylindrical, the apical half enlarged. Ejaculatory pore terminal, on a verge. Verge relatively large (1.0 mm), terminal, dorso-laterally compressed, back-pointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 8 terminal papillae (Fig. 3b). Dorsal pilaster long (11 mm) and broad (mid-width 1.4 mm), and superficially resembling a stack of tightly appressed tongue-like lappets with smooth edges. Basal one-fourth to one-third of the penis smooth with random folds; upper half uniformly sculpted with 25–35 adjacent, generally unmerging, equilateral columns of distinct, equal-sized pus-

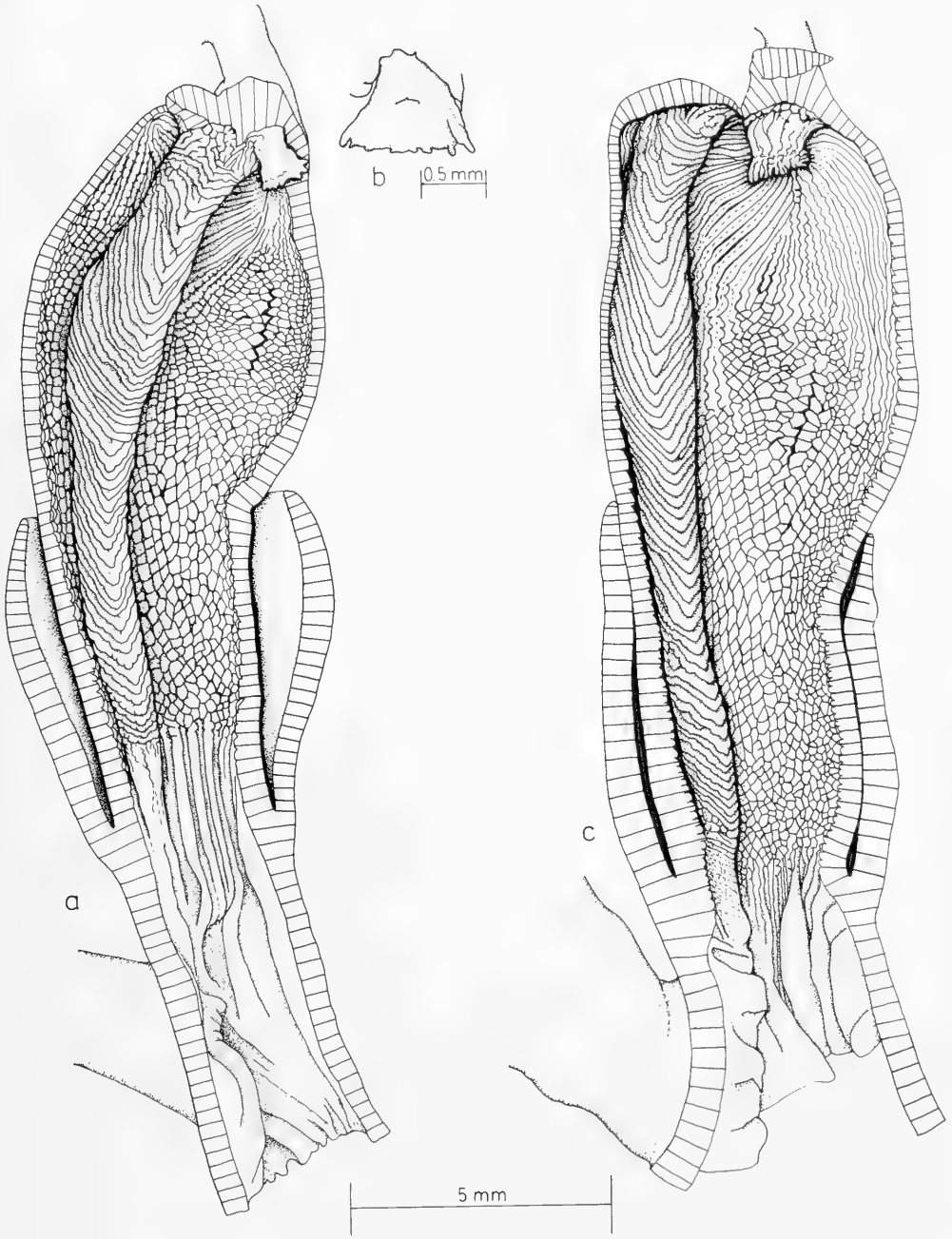

FIG. 3. Opened uneverted penial tubes. **a.** *Neohelix alleni alleni* (Sampson, 1883). FMNH 214911 #12 (also dissected #11, 13; and 7 other populations—see Appendix B). **b.** Reverse side of verge of a, showing opening of vas deferens. **c.** *Neohelix alleni fuscolabris* (Pilsbry, 1903). FMNH uncat. #1 (also dissected #2; FMNH uncat. #7, 11, 15). These two subspecies have probably been separated by the Mississippi River Valley for at least 20,000 years but show little difference in penial morphology.

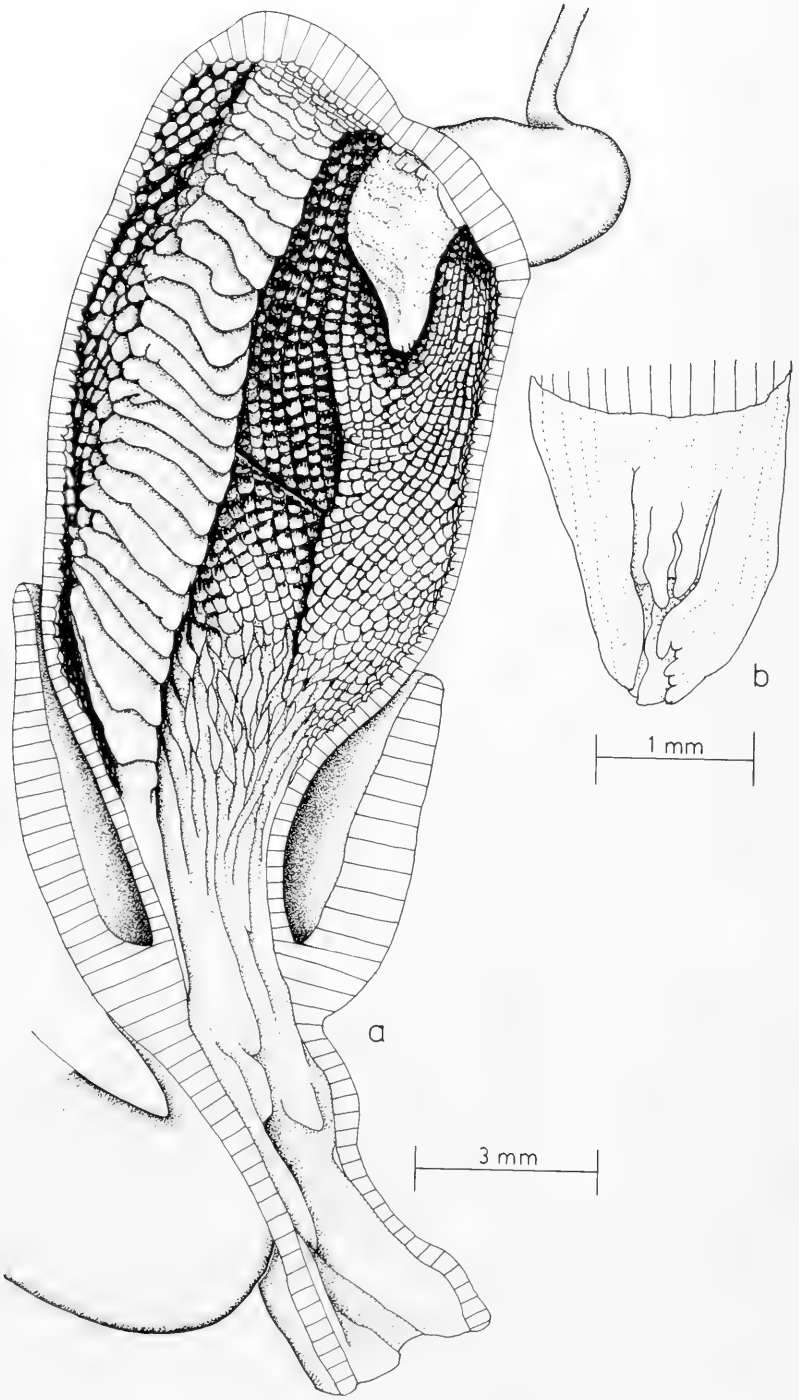

FIG. 4. Opened uneverted penial tube. **a.** *Neohelix major* (BInney, 1837). FMNH 214930 #6 (also dissected #7, 8; and other populations—see systematic section). **b.** Detail of the reverse side of verge of a, showing opening of vas deferens.

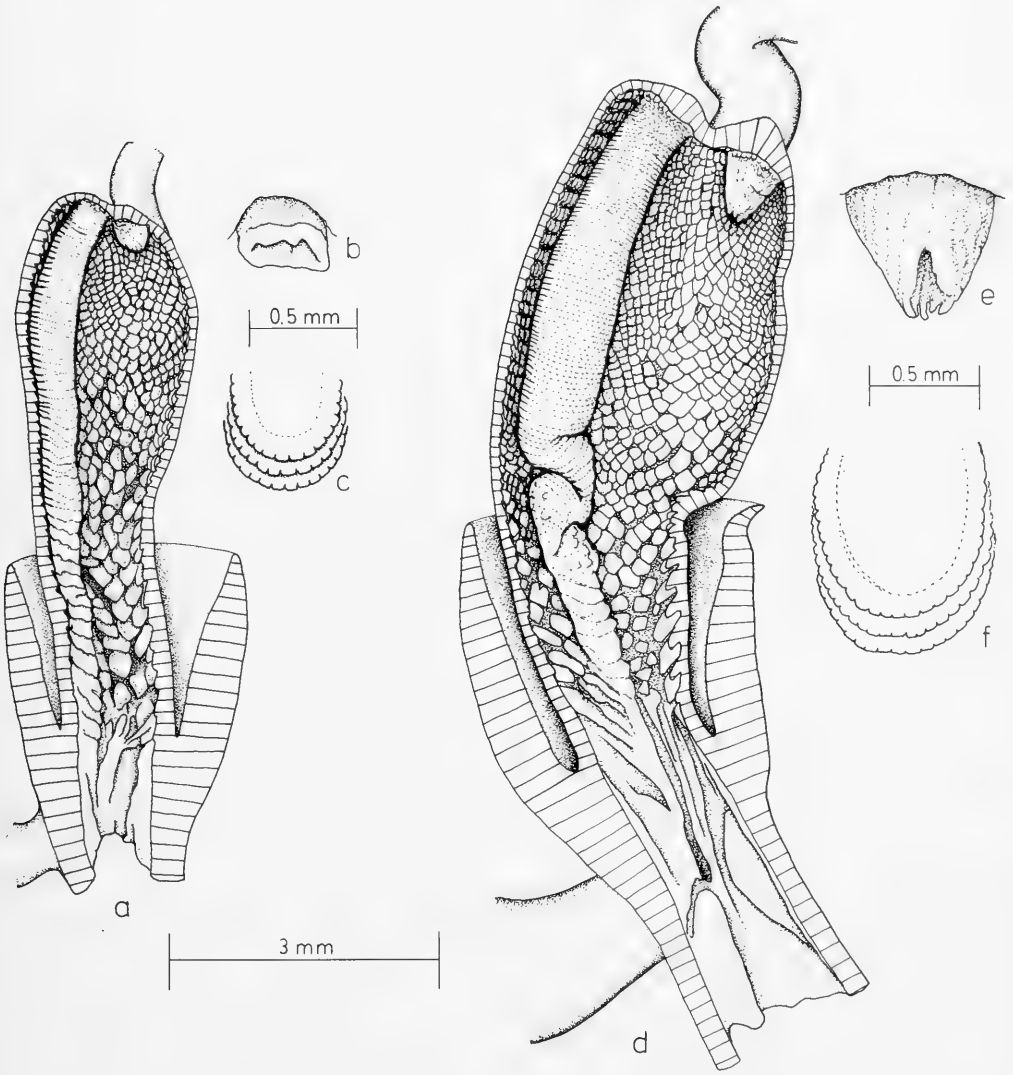

FIG. 5. Opened uneverted penial tubes. **a.** *Neohelix lioderma* (Pilsbry, 1902). FMNH 214844 #A (also dissected #9 and #B, C). **b.** Reverse of verge of a, showing opening of vas deferens. **c.** Detail of 3 lappets from center of pilaster of a, showing substructure suggesting laterally fused pustule. **d.** *Neohelix divesta* (Gould, 1848). FMNH 214813 #1 (also dissected #7, 8, 10; FMNH 176089). **e.** Reverse of verge of d, showing opening of vas deferens. **f.** Detail of 3 central lappets of pilaster of d, showing substructure suggesting laterally fused pustules.

tules radiating from the pore region. Sheath enclosing less than half of the upper, sculpted region of the penis.

Neohelix dentifera (Binney, 1837)—Dissections: 6 from 2 populations. Fig. 2a-c. Length 12 mm. Shape cylindrical, the apical half enlarged. Ejaculatory pore terminal, on a verge. Verge moderate in size (length 0.9 mm), terminal, dorso-laterally compressed, back-pointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 6 terminal papillae. Dorsal pilaster long (7 mm) and broad (mid-width mm), and superficially resembling a stack of thin, plate-like lappets with edges comprised

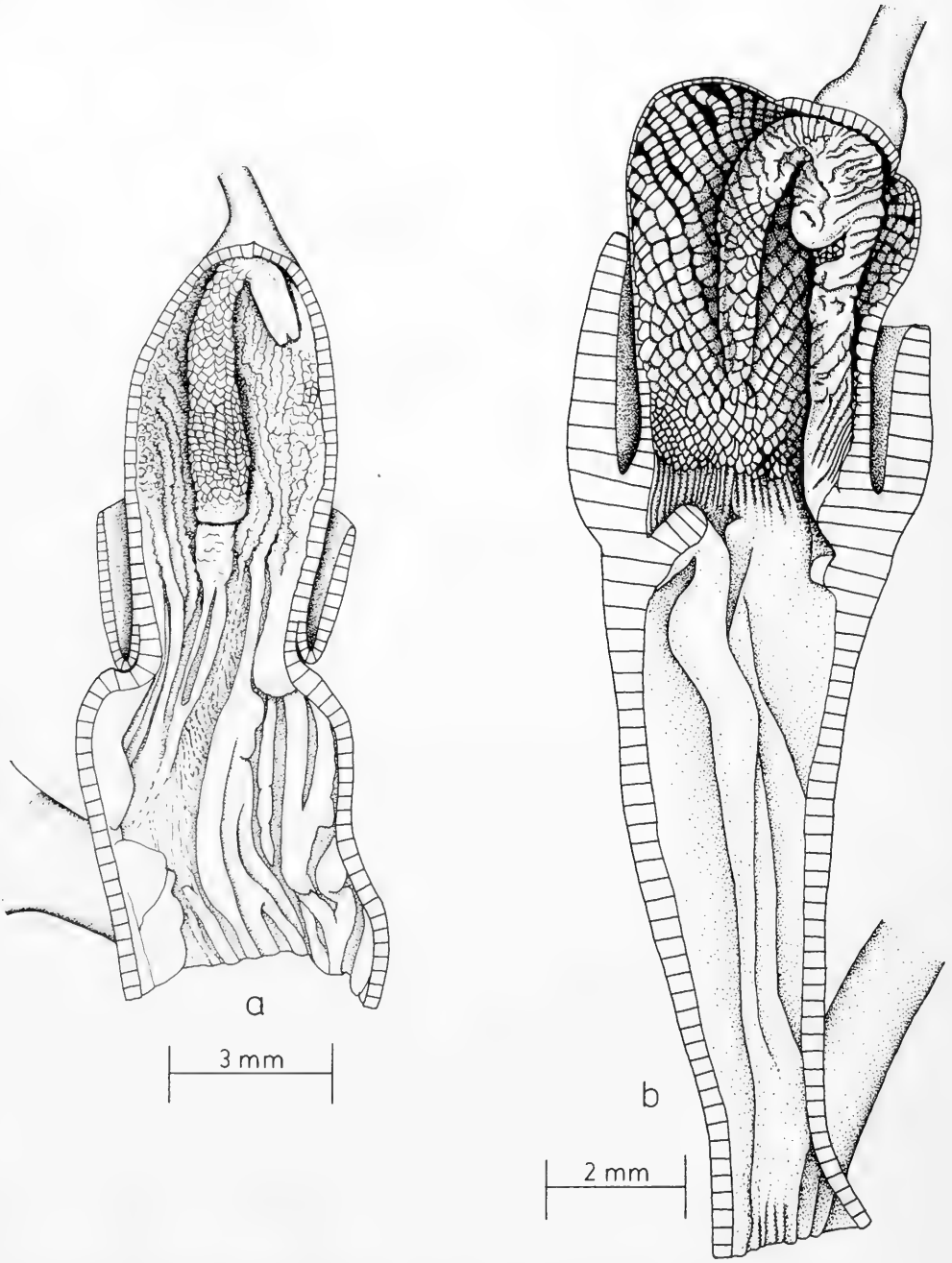

FIG. 6. Opened uneverted penial tubes. **a.** *Webbhelix multilineata* (Say, 1821). FMNH 214848 #2 (also dissected FMNH 214849 #1, 5, A; Hubricht 48600 #A, B, C). **b.** *Neohelix solemi* Emberton, new species. FMNH 214936 #1 (also dissected 13 other populations—see Appendix B).

of equal, bi-lobed units (Fig. 2b). Basal one-fourth of the penis smooth with random folds; upper half uniformly sculpted with 25–35 adjacent, generally unmerging, equilateral columns of distinct, approximately equal-sized, lobed pustules (Fig. 2c), radiating from the

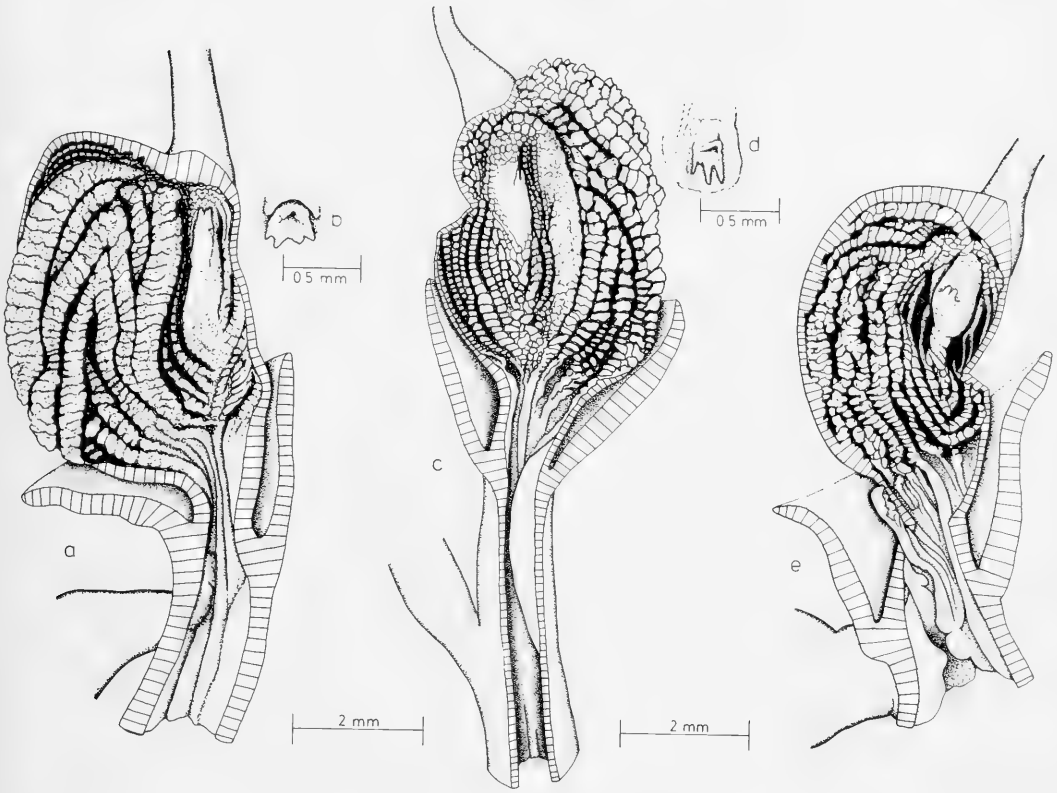

FIG. 7. Opened uneverted penial tubes. **a.** *Xolotrema denotata* (Férussac, 1821). FMNH 214806 #6 (also dissected #1, 2). **b.** Reverse of verge of a. **c.** *Xolotrema obstricta* (Say, 1821). FMNH 214854 #9 (also dissected #1). **d.** Reverse of verge of c. **e.** *Xolotrema caroliniensis* (Lea, 1834). FMNH 171142 #A (also dissected #B).

pore region. Sheath enclosing one half to two-thirds of the upper, sculpted region of the penis.

Neohelix divesta (Gould, 1848)—Dissections: 4 from 1 population. Fig. 5d-f. Length 10 mm. Shape cylindrical, the apical half enlarged. Ejaculatory pore terminal, on a verge. Verge moderate in size (length 0.6 mm), terminal, dorso-laterally compressed, back-pointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 6 terminal papillae (Fig. 5e). Dorsal pilaster long (7 mm) and broad (mid-width 0.8 mm), and superficially resembling a stack of thin, plate-like lappets with regularly indented edges (Fig. 5f). Basal one-fourth of the penis smooth with random folds; upper half uniformly sculpted with 25–35 adjacent, generally unmerging, equilateral columns of distinct, approximately equal-sized, lobed pustules, radiating from the pore region. Sheath enclosing less than half of the upper, sculpted region of the penis.

Neohelix lioderma (Pilsbry, 1902)—Dissections: 4 from 1 population. Fig. 5a–c. Length 7 mm. Shape cylindrical, the apical half enlarged. Ejaculatory pore terminal, on a verge. Verge (shown partially inverted in Fig. 5a–b) moderate in size (0.3 mm), terminal, dorso-laterally compressed, back-pointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 6 terminal papillae (not visible in Fig. 5b). Dorsal pilaster long (6 mm) and broad (mid-width 0.5 mm), and superficially resembling a stack of thin, plate-like lappets with regularly indented edges (Fig. 5c). Basal one-fourth of the penis smooth with random folds; upper half uniformly sculpted with 25–35 adjacent, generally unmerging, equilateral columns of

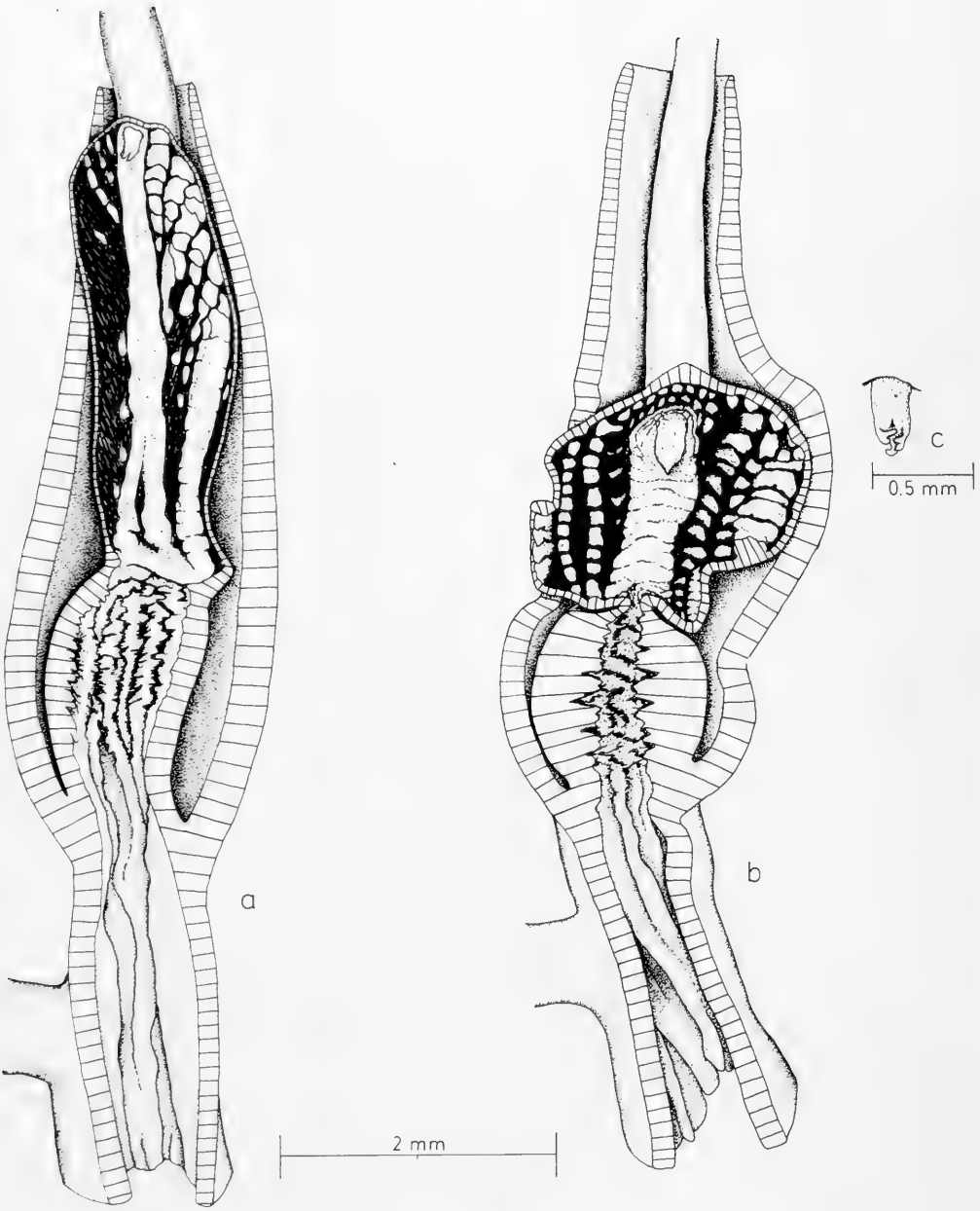

FIG. 8. Opened uneverted penial tubes. **a.** *Xolotrema fosteri* (F. C. Baker, 1932). FMNH 214817 #A (also dissected #B, C, D, E; FMNH 214819 #19). **b.** *Xolotrema occidentalis* (Pilsbry & Ferriss, 1907). FMNH 214856 #5. **c.** Reverse side of verge of b, showing opening of vas deferens.

distinct, approximately equal-sized, lobed pustules, radiating from the pore region. Sheath enclosing less than half of the upper, sculpted region of the penis.

Neohelix major (Binney, 1837)—Dissections: 10 from 5 populations. Fig. 4. Length: 23 mm. Shape cylindrical, the apical half enlarged. Ejaculatory pore terminal, on a

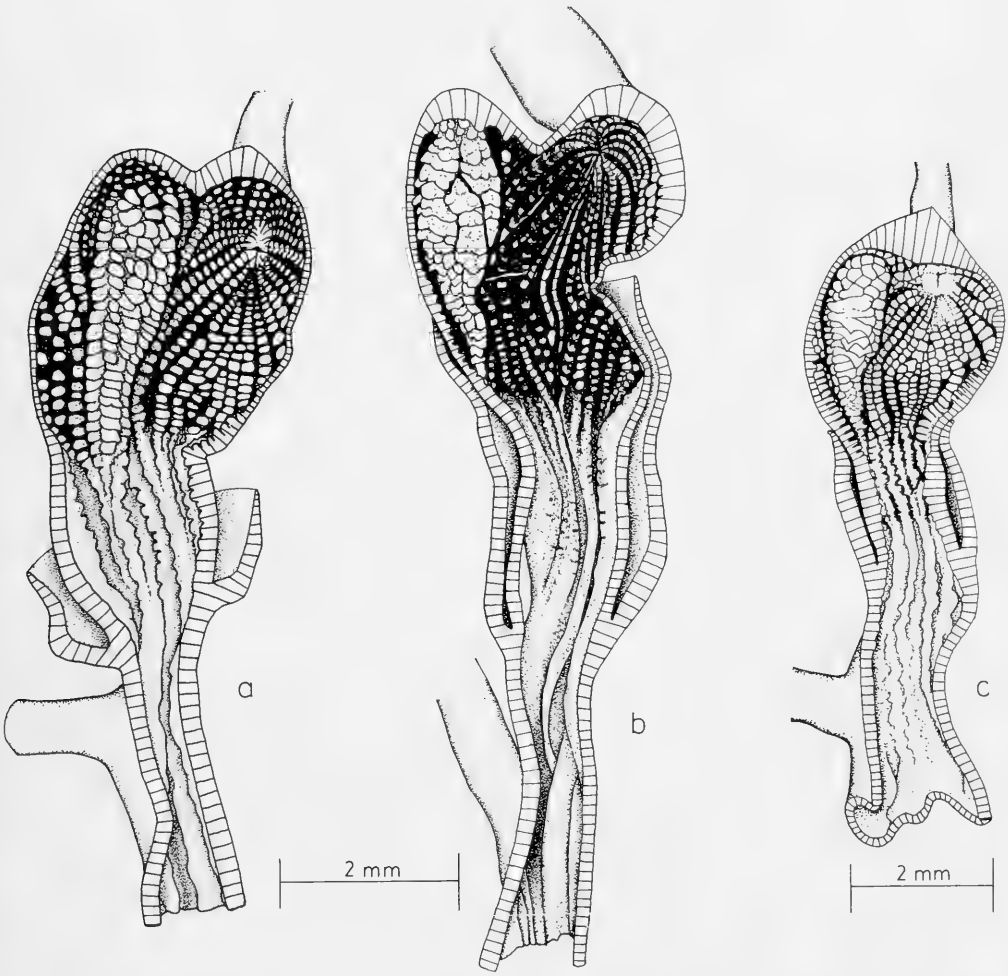

FIG. 9. Opened uneverted penial tubes. **a.** *Triodopsis vulgata* Pilsbry, 1940. FMNH 214884 #1 (also dissected FMNH 214883 #2, 3; FMNH 214885 #1, 2, 3, 4). **b.** *Triodopsis picea* Hubricht, 1958. FMNH 214860 #14 (also dissected #4). **c.** *Triodopsis claibornensis* Lutz, 1950. FMNH 214800 #18 (also dissected #5: has broader pilaster).

verge. Verge very large (length 3.0 mm), terminal, dorso-laterally compressed, backpointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 12 terminal papillae (Fig. 2b). Dorsal pilaster long (12 mm) and broad (mid-width 2.2 mm), and superficially resembling a stack of tongue-like lappets with edges pronouncedly convex and irregularly wavy, with no regularly-spaced indentations. Basal half of the penis smooth with random folds; upper half uniformly sculpted with 25–35 adjacent, generally unmerging, equilateral columns of distinct, equal-sized pustules radiating from the pore region. Sheath enclosing less than half of the upper, sculpted region of the penis.

Neohelix solemi Emberton, new species—Dissections: 24 from 13 populations. Fig. 6b. Length: 17 mm. Shape cylindrical, the apical half enlarged. Ejaculatory pore dorsally subterminal, on a tiny verge which lies on the apex of a thick, fleshy protuberance. Verge minute (length 0.1 mm), dorso-laterally compressed, backpointing, with a ventrally subterminal pore and sculpted with surface cords continuing into 6–8 terminal papillae. Dorsal pilaster relatively short (3 mm) and narrow (mid-width 0.5 mm), merging terminally with

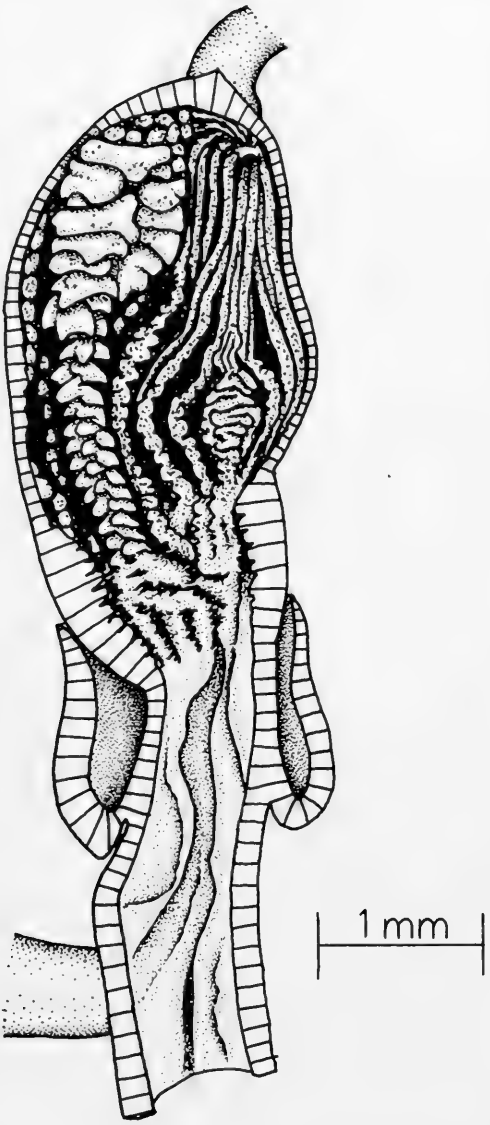

FIG. 10. Opened uneverted penial tube. *Triodopsis fraudulenta* (Pilsbry, 1894). FMNH 214822 #6 (also dissected #8: has more and smaller parts in thickest part of pilaster, with wall pustules more pronounced).

the fleshy protuberance, and superficially resembling an indistinct stack of indistinct tongue-like lappets with variously shaped edges. Ventral wall bearing one to three fold-like pilasters, sculptured no differently than the adjacent penial wall. Basal three-fifths of the penis smooth with random folds; upper two-fifths uniformly sculpted with 25–35 adjacent, generally unmerging, equilateral columns of distinct, equal-sized pustules radiating from the pore region. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis alabamensis (Pilsbry, 1902)—Dissections: 2 from 1 population. Fig. 16a. Length: 7 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (1 mm) and tapered proximally (mid-width 0.4 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis anteridon (Pilsbry, 1940)—Dissections: 3 from 2 populations. Fig. 14b. Length: 7 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (1.5 mm) and tapered proximally (mid-width 0.6 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis burchi Hubricht, 1950—Dissections: 3 from 1 population. Fig. 11a. Length: 8 mm. Shape like a baseball bat. Ejaculatory pore terminal. Verge absent. Dorsal pilaster long (3 mm) and broad (mid-width 1.4 mm), consisting of abutting, unequaly-sized polygons, each covered with knob-like pustules about twice as large as the wall-pustules. Basal half of the penis smooth with random folds and slight circular corrugations; upper half sculpted with 15–20 columns of

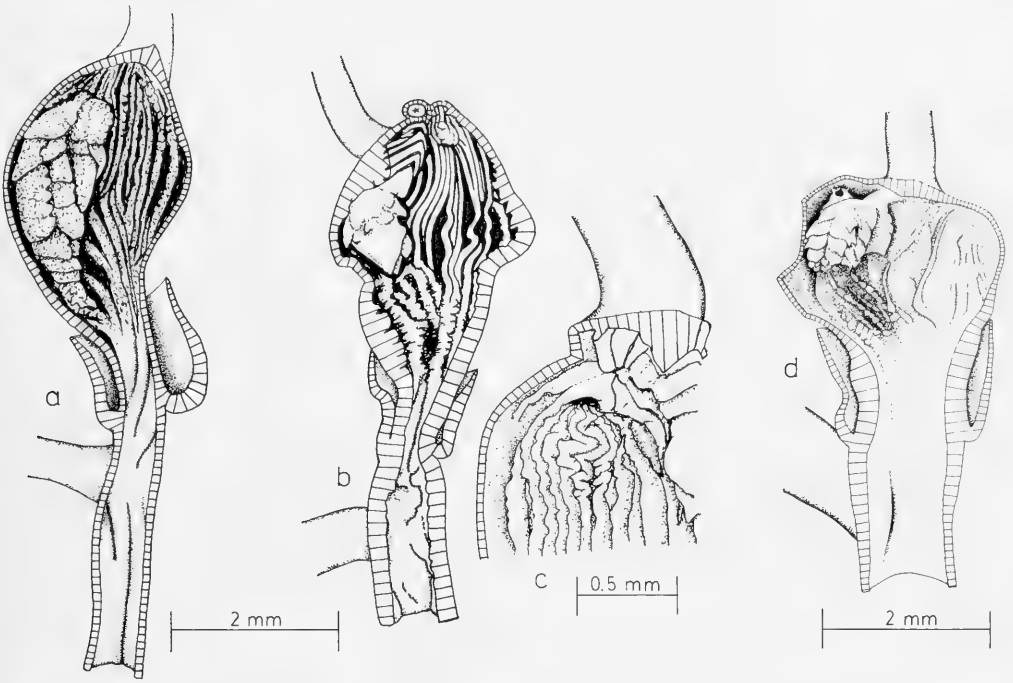

FIG. 11. Opened uneverted penial tubes. **a.** *Triodopsis burchi* Hubricht, 1950. FMNH 214797 #3 (also dissected #5, 12). **b.–c.** *Triodopsis tennesseensis* (Walker & Pilsbry, 1902). **b.** FMNH 214864 #15 (also dissected #13, 14). **c.** Area around opening of vas deferens in #14, showing lack of verge. **d.** *Triodopsis complanata* (Pilsbry, 1898). Hubricht 17932 #C (also dissected #A, B).

equal-sized pustules radiating directly from the pore, the ventral-most columns with pustules indistinct, appearing almost smooth. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis claibornensis Lutz, 1950—Dissected 2 from 1 population. Fig. 9c. Length: 8 mm. Shape like a baseball bat. Ejaculatory pore ventrally subterminal, about one-fifth-way from the penial apex in the upper, sculpted region. Verge absent. Dorsal pilaster long (2.3 mm) and broad (mid-width 0.8 mm), covered with knob-like pustules all about twice as large as the wall-pustules. Basal half of the penis smooth with random folds and slight circular corrugations; upper half sculpted with 15–20 columns of distinct, equal-sized pustules radiating directly from the pore. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis complanata (Pilsbry, 1898)—Dissections: 3 from 1 population. Fig. 11d (a contracted specimen). Length: 5 mm. Shape like a baseball bat. Ejaculatory pore terminal. Verge absent. Dorsal pilaster short (1 mm) and broad (mid-width 0.8 mm), consisting of a solid mass bearing three tiers of long, sharp spurs. Basal third of the penis smooth with random folds and slight circular corrupations; upper two-thirds sculpted with 15–20 columns radiating directly from the pore, the dorsal columns bearing indistinct, equal-sized pustules, and the ventral columns completely smooth. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis cragini Call, 1886—Dissections: 2 from 1 population. Fig. 13b. Length: 8 mm. Shape like a needle. Ejaculatory pore terminal. Verge absent. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.3 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal third of the penis smooth with random folds; middle third with slight circular corrugations; upper third sculpted with equilateral, widely sepa-

FIG. 12. Opened uneverted penial tube. *Triodopsis platysayoides* (Brooks, 1933). FMNH 214861 #1 (also dissected #2; examined Hubricht 11860 [illustrated in Solem, 1976]).

rated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal third of the penis.

Triodopsis discoidea (Pilsbry, 1904)—Dissections: 1 from 1 population. Fig. 14d. Length: 6 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, two-fifths-way from the apex in the upper, sculpted region. Verge absent. A large, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.5 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis fallax (Say, 1825)—Dissections: 3 from 1 population. Fig. 14b (a contracted specimen). Length: 7 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.7 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis fraudulenta (Pilsbry, 1894)—Dissected 2 from 1 population. Fig. 10. Length: 6 mm. Shape like a baseball bat. Ejaculatory pore ventrally subterminal, about one-fifth-way from the penial apex in the upper, sculpted region. Verge absent. Dorsal pilaster long (3 mm) and broad (mid-width 0.8 mm), consisting of nesting horeshoe-shaped units covered with knob-like pustules about twice as large as the wall-pustules. Basal third of the penis smooth with random folds and slight circular corrugations; upper two-thirds sculpted with 15–20 columns radiating directly from the pore, the dorsal columns bearing distinct, equal-sized pustules, and the ventral columns smooth, the ventral-most merging basally into a complexly ridged protuberance. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis fulciden Hubricht, 1952—Dis-

FIG. 13. Opened uneverted penial tubes. **a.** *Triodopsis vultuosa* (Gould, 1848). FMNH 214887 #A (also dissected #13: no trace of a verge; vas deferens opening terminal). **b.** *Triodopsis cragini* Call, 1886. FMNH 214803 #18 (also dissected #3: more pronounced pilaster and no sign of verge). **c.** *Triodopsis henriettae* (Mazÿck, 1877). FMNH 214824 #1: pilaster seemed partly deteriorated, with structure vague.

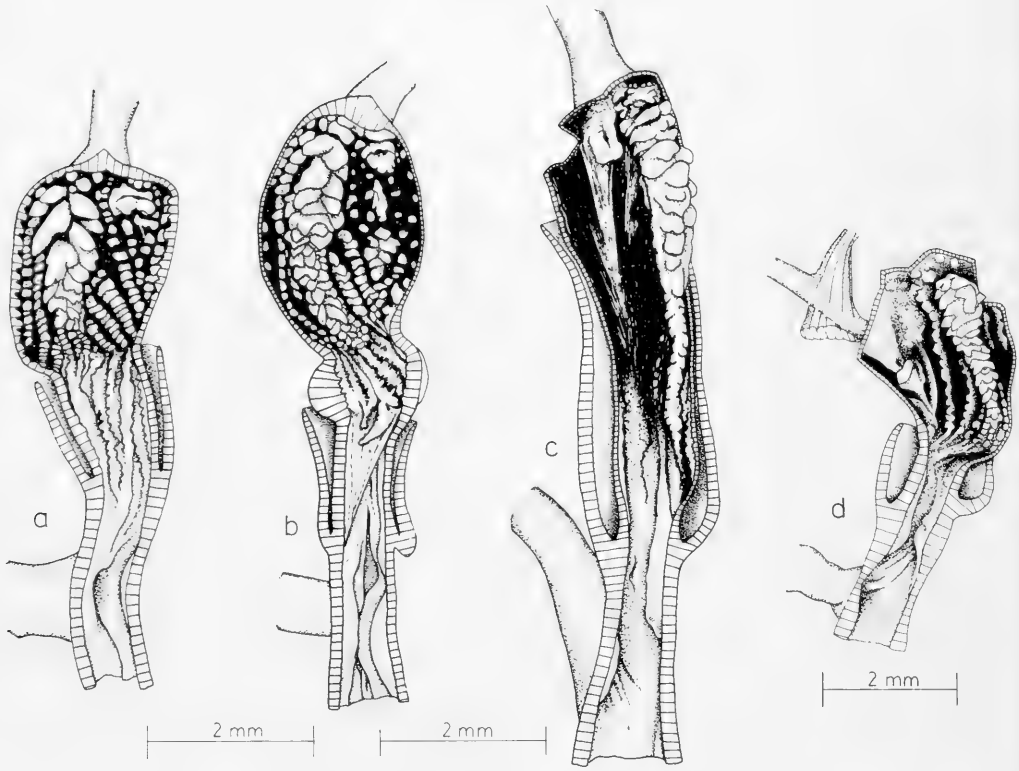

FIG 14. Opened uneverted penial tubes. **a.** *Triodopsis tridentata* (Say, 1816). FMNH 214876 (sympatric with *Triodopsis juxtidens*) #32 (also dissected 7 other populations——see Appendix C). **b.** *Triodopsis anteridon* (Pilsbry, 1940). FMNH 214796 #18 (also dissected FMNH 214793 #13, 14). **c.** *Triodopsis juxtidens* (Pilsbry, 1894). FMNH 214841 #5 (also dissected #10; FMNH 214838 #1, 2, 3; FMNH 214839 #4; FMNH 214842 #5, 6). **d.** *Triodopsis discoidea* (Pilsbry, 1904). FMNH 214811 #5.

sections: 1. Fig. 18b. Length: 3 mm. Shape like a baseball bat. Ejaculatory pore terminal. Verge absent. Dorsal pilaster two-thirds the length of the sculpted region of the penis (1 mm) and tapered proximally (mid-width 0.3 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fifth of the penis smooth with random folds: middle fifth with thick muscular walls bearing slight circular corrugations; upper three-fifths sculpted with 15–20 columns of equal-sized pustules radiating directly from the pore, the ventral columns with pustules indistinct, and the ventralmost columns merging basally. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis henriettae (Mazyck, 1877)—Dissections: 2 from 1 population. Fig. 13c. Length: 9 mm. Shape like a needle. Ejaculatory pore terminal. Verge absent. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.2 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal third of the penis smooth with random folds; middle third with slight circular corrugations; upper third sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal third of the penis.

Triodopsis hopetonensis (Shuttleworth, 1852)—Dissections: 3 from 1 population. Fig. 15a. Length: 7 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath

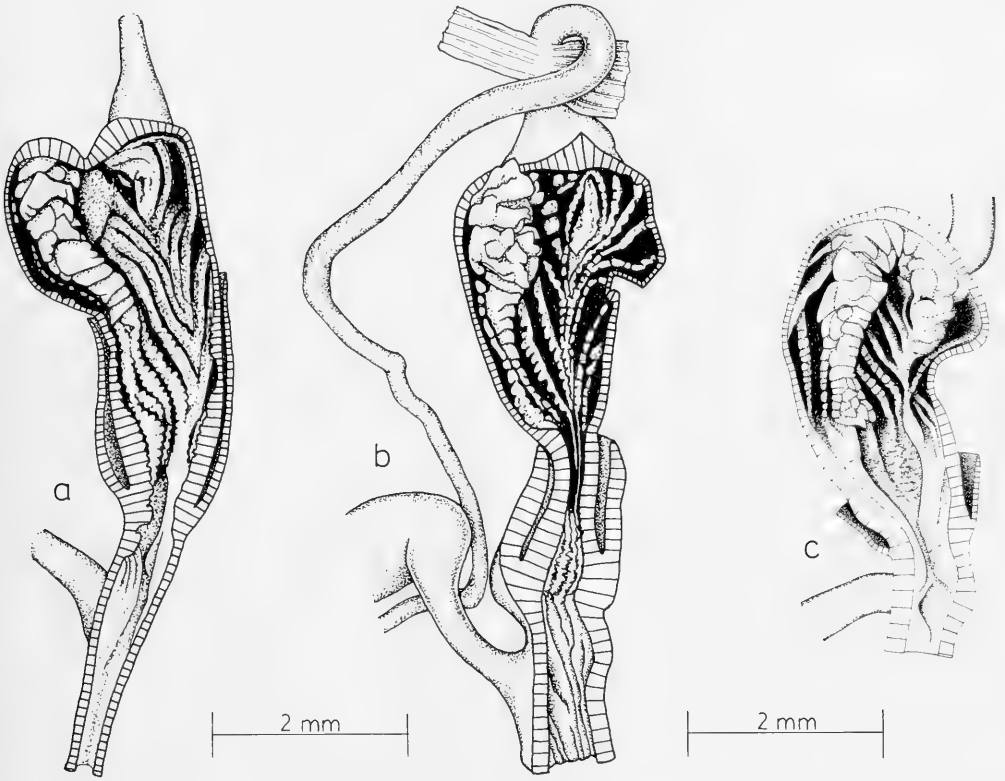

FIG. 15. Opened uneverted penial tubes. **a.** *Triodopsis hopetonensis* (Shuttleworth, 1852). FMNH 214827 #A (also dissected #15, 25). **b.** *Triodopsis palustris* Hubricht, 1958. FMNH 214857 #15 (also dissected #4, 5). **c.** *Triodopsis obsoleta* (Pilsbry, 1894). Hubricht 10300 #C (also dissected #A, B).

the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.4 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis juxtidens (Pilsbry, 1894)—Dissections: 6 from 3 populations. Fig. 14c. Length: 8 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, two-fifths-way from the apex in the upper, sculpted region. Verge absent. A large, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (3 mm) and tapered proximally (mid-width 0.5 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing about half of the upper, sculpted region of the penis.

Triodopsis messana Hubricth, 1952—Dissections: 3 from 1 population. Fig. 16b. Length: 8 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (1 mm) and tapered proximally (mid-width 0.8 mm), con-

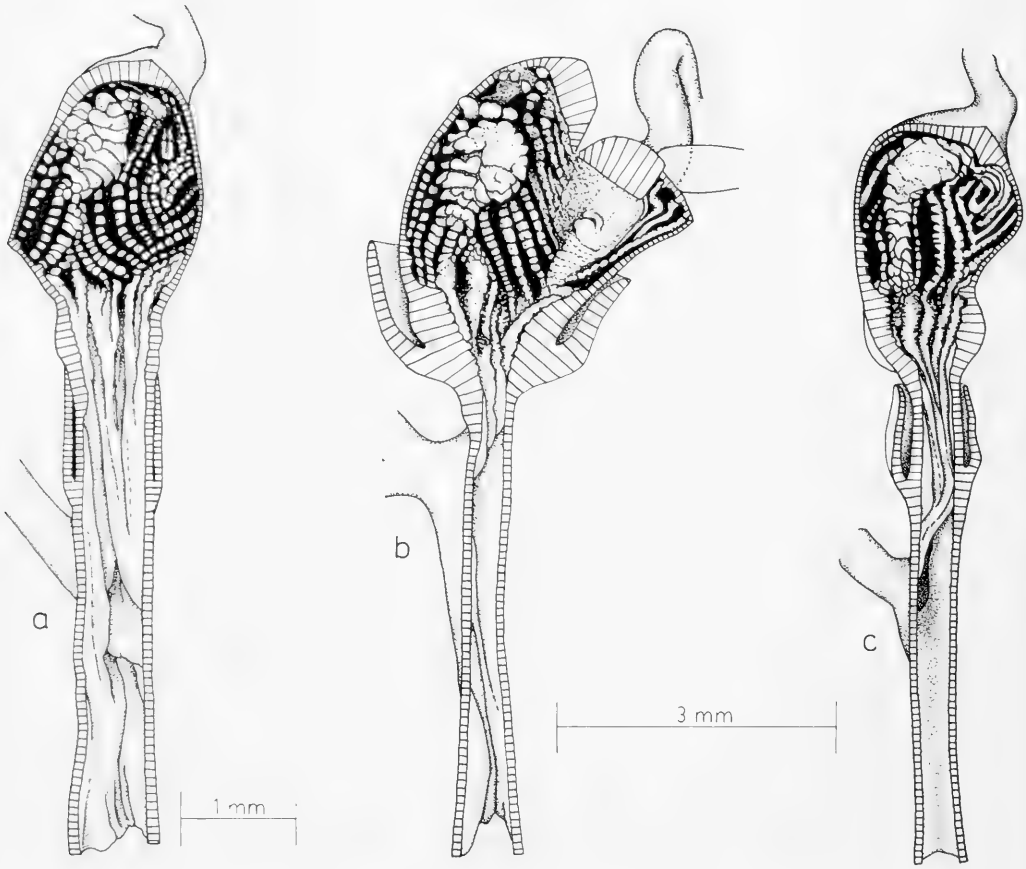

FIG. 16. Opened uneverted penial tubes. **a.** *Triodopsis alabamensis* (Pilsbry, 1902). FMNH 214791 #4 (also dissected #2: pilaster smaller and more lobular). **b.** *Triodopsis messana* Hubricht, 1952. FMNH 214846 #6 (also dissected #1 and #5, both with sculpture more effaced and with wall less tightly contracted). **c.** *Triodopsis vannostrandi* (Bland, 1875). FMNH 214880 #8 (also dissected #1 and #12, both with wall sculpture more effaced).

sisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis neglecta (Pilsbry, 1899)—Dissections: 2 from 1 population. Fig. 18a. Length: 5 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, two-fifths-way from the apex in the upper, sculpted region. Verge absent. A large, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (1 mm) and tapered proximally (mid-width 0.5 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis obsoleta (Pilsbry, 1894)—Dissections: 3 from 1 population. Fig. 15c (a contracted specimen). Length: 5 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in

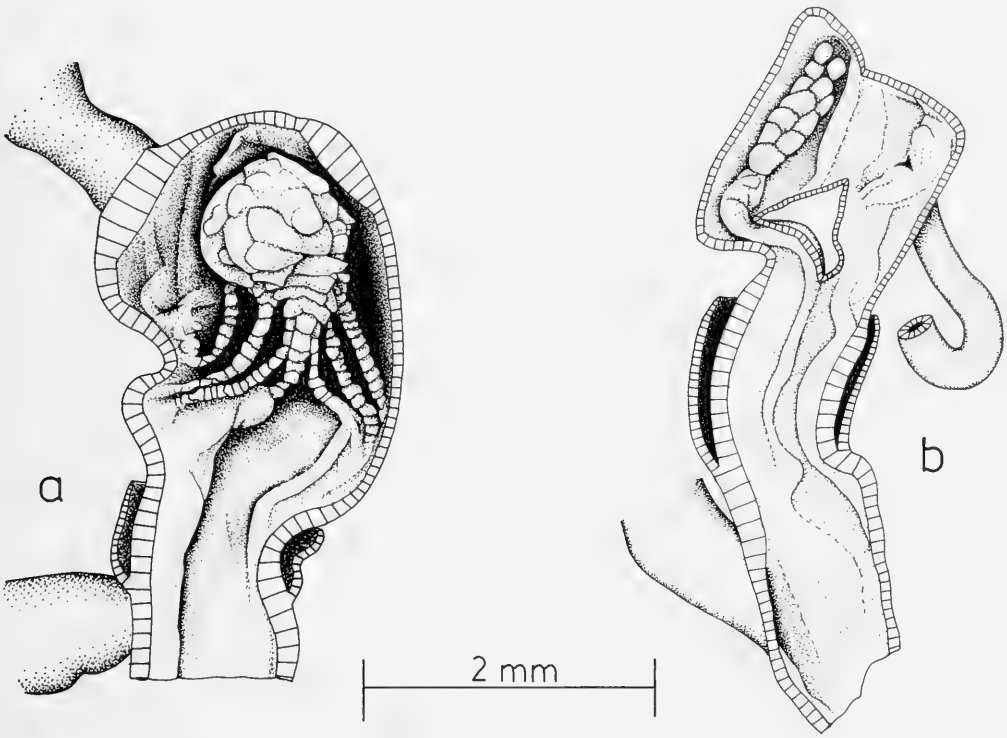

FIG. 17. Opened uneverted penial tubes. **a.** *Triodopsis fallax* (Say, 1825). Hubricht 10209 #C (also dissected #A, B). **b.** *Triodopsis soelneri* (Henderson, 1907). ANSP A2318 (alcohol-preserved soft parts pulled from shells of ANSP 93545) #B (also dissected #A, C).

the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (1 mm) and tapered proximally (mid-width 0.5 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis palustris Hubricht, 1958—Dissections: 3 from 1 population. Fig. 15b. Length: 6 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.7 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis pendula Hubricht, 1952—Dissections: 1 from 1 population. Fig. 18c. Length: 5 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, two-fifths-way from the apex in the upper, sculpted region. Verge absent. A large, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.4 mm), consisting of abutting irregularly sized and

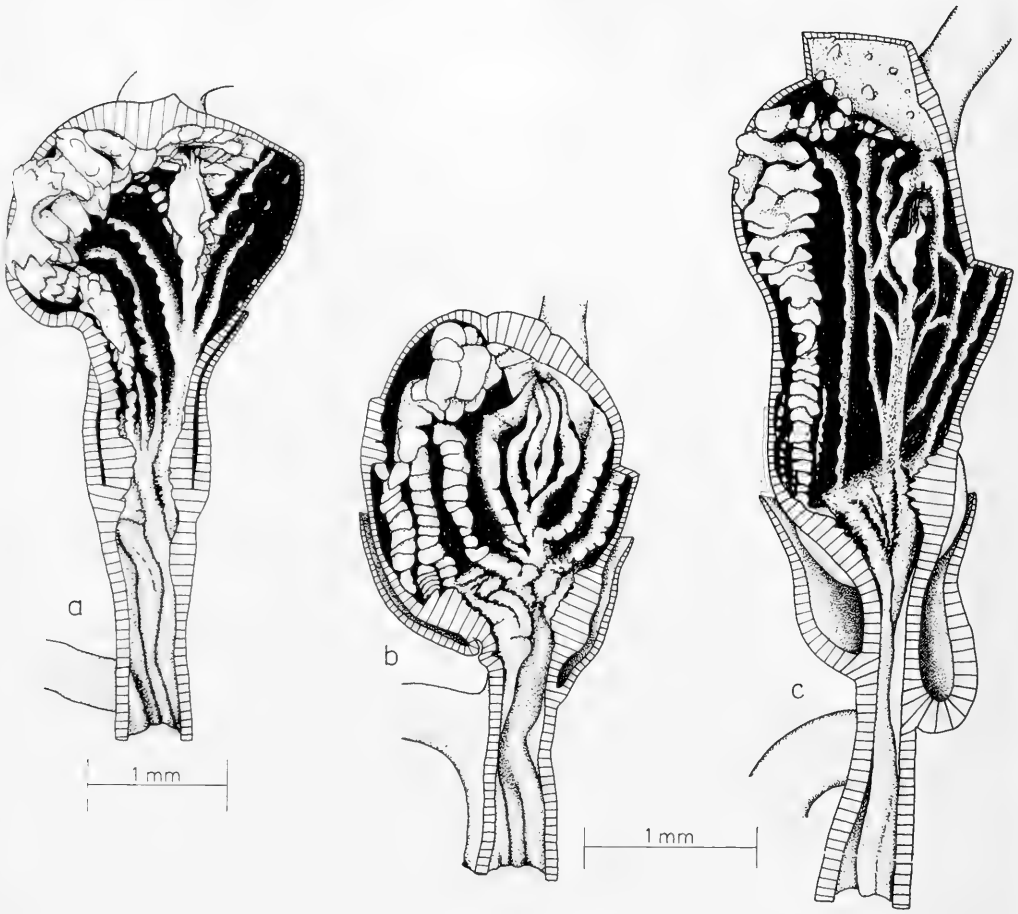

FIG. 18. Opened uneverted penial tubes. **a.** *Triodopsis neglecta* (Pilsbry, 1899). FMNH 214850 #2 (also dissected #5: no verge). **b.** *Triodopsis fulciden* Hubricht, 1952. FMNH 214823 #3. No verge; opening of vas deferens a simple hole. **c.** *Triodopsis pendula* Hubricht, 1952. FMNH 214859 #8.

shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing about half of the upper, sculpted region of the penis.

Triodopsis picea Hubricht, 1958—Dissections: 2 from 1 population. Fig. 9b. Length: 10 mm. Shape like a baseball bat. Ejaculatory pore ventrally subterminal, about one-fifth-way from the penial apex in the upper, sculpted region. Verge absent. Dorsal pilaster long (3 mm) and broad (mid-width 0.9 mm), consisting of nesting horseshoe-shaped untis covered with knob-like pustules about twice as large as the wall-pustules. Basal half of the penis smooth with random folds and slight circular corrugations; upper half sculpted with 15–20 columns of distinct, equal-sized pustules radiating directly from the pore. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis platysayoides (Brooks, 1933)—Dissections: 3 from 1 or 2 populations. Fig. 12. Length: 13 mm. Shape like a baseball bat. Ejaculatory pore terminal. Verge absent. Dorsal pilaster long (7 mm) and very broad (mid-width 1.7 mm), consisting of two interdigitating columns of rectangular boxes,

each covered with knob-like pustules about twice as large as the wall-pustules. Basal third of the penis smooth with random folds; upper two-thirds sculpted with equilateral, widely spaced columns of distinct, equal-sized pustules, merging ventrally into 10–12 obtuse V-shapes. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis rugosa Brooks & MacMillan, 1940—Dissections: 2 from 1 population. Not illustrated but similar to Fig. 18b. Length: not measured. Shape like a baseball bat. Ejaculatory pore terminal. Verge absent. Dorsal pilaster two-thirds the length of the sculpted region of the penis and tapered proximally, consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fifth of the penis smooth with random folds; middle fifth with thick muscular walls bearing slight circular corrugations; upper three-fifths sculpted with 15–20 columns of equal-sized pustules radiating directly from the pore, the ventral columns with pustules indistinct, and the ventralmost columns merging basally. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis soelneri (Henderson, 1907)—Dissections: 3 from 1 population. Fig. 17b. Length: 5 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (1 mm) and tapered proximally (mid-width 0.3 mm), consisting of abutting irregularly sized and shaped polygons, each smooth and without spurs. Basal half of the penis smooth with random folds; upper half smooth with dorsal traces of equilateral, widely separated columns. Sheath enclosing only the basal half of the penis.

Triodopsis tennesseensis (Walker & Pilsbry, 1902)—Dissections: 3 from 1 population. Fig. 11b–c. Length: 6 mm. Shape like a baseball bat. Ejaculatory pore terminal. Verge absent. Dorsal pilaster short (1 mm) and broad (mid-width 0.8 mm), consisting of a solid mass bearing three tiers of long, sharp spurs. Basal third of the penis smooth with random folds and slight circular corrugations; upper two-thirds sculpted with 15–20 columns radiating directly from the pore, the dorsal columns bearing indistinct, equal-sized pustules, and the ventral columns completely smooth. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis tridentata (Say, 1816)—Dissections: 10 from 8 populations. Fig. 14a. Length: 6 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.7 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis vannostrandi (Bland, 1875)—Dissections: 3 from 1 population. Fig. 16c. Length: 8 mm. Shape like a mace. Ejaculatory pore ventrally subterminal, one-fourth-way from the apex in the upper, sculpted region. Verge absent. A small, smooth, fleshy peduncle present just beneath the pore. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.4 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal fourth of the penis smooth with random folds; middle fourth with slight circular corrugations; upper half sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal half of the penis.

Triodopsis vulgata (Pilsbry, 1940)—Dissects: 7 from 3 populations. Fig. 9a. Length: 9 mm. Shape like a baseball bat. Ejaculatory pore ventrally subterminal, about one-fifth-way from the penial apex in the upper, sculpted region. Verge absent. Dorsal pilaster long (3 mm) and broad (mid-width 0.9 mm), covered with knob-like pustules all about twice as large as the wall-pustules. Basal half of the penis smooth with random folds and slight circular corrugations; upper

half sculpted with 15–20 columns of distinct, equal-sized pustules radiating directly from the pore. Sheath enclosing less than half of the upper, sculpted region of the penis.

Triodopsis vultuosa (Gould, 1848)—Dissections: 2 from 1 population. Fig. 13a. Length: 7 mm. Shape like a needle. Ejaculatory pore terminal. Verge absent. Dorsal pilaster two-thirds the length of the sculpted region of the penis (2 mm) and tapered proximally (mid-width 0.2 mm), consisting of abutting irregularly sized and shaped polygons, each bearing one to three short, blunt spurs. Basal third of the penis smooth with random folds; middle third with slight circular corrugations; upper third sculpted with equilateral, widely separated columns of equal-sized pustules, merging ventrally into 5–7 acute V-shapes. Sheath enclosing only the basal third of the penis.

Webbhelix multilineata (Say, 1821)—Dissections: 7 from 3 populations. Fig. 6a. Length: 14 mm. Shape cylindrical, the apical half enlarged. Ejaculatory pore terminal, on a verge. Verge large (1.2 mm), terminal, dorsolaterally compressed, backpointing, with a ventrally subterminal pore, smoothly sculpted, and bearing two broad, prominent terminal papillae. Dorsal pilaster short (5 mm) and broad (mid-width 1.0 mm), proximally truncated, and covered with small, uniform, pointed pustules. Basal two-thirds of the penis smooth with random folds; upper one-third uniformly sculpted with 25–35 adjacent, generally unmerging, equilateral columns of distinct, equal-sized pustules radiating from the pore region; the pustules are indistinct on the basal two-thirds of these columns. Sheath enclosing less than half of the upper, sculpted region of the penis.

Xolotrema caroliniensis (Lea, 1834)—Dissections: 2 from 1 population. Fig. 7e. Length: 8 mm. Shaped like a pear. Ejaculatory pore ventrally subterminal, about one-third-way from the penial apex in the upper, scupted region, and on a verge. Verge small (0.2 mm), wider than long, ventrally subterminal on a slight prominence, dorso-laterally compressed, forward-pointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 4 narrow terminal papillae. Dorsal pilaster indistinct from the columns of wall pustules, and consisting of 5 broad, nested A-shapes. Basal one-third of the penis smooth with random folds; upper two-thirds sculpted with slightly separated columns of cuboidal, rough-surfaced pustules, enlarging and merging and ventrally into 6–10 tapered U-shapes. Sheath enclosing less than half of the upper, sculpted region of the penis.

Xolotrema denotata (Férussac, 1821)—Dissections: 3 from 1 population. Fig. 7a–b. Length: 9 mm. Shaped like a pear. Ejaculatory pore ventrally subterminal, about one-third-way from the penial apex in the upper, scupted region, and on a verge. Verge small (0.2 mm), wider than long, ventrally subterminal on a slight prominence, dorso-laterally compressed, forward-pointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 4 narrow terminal papillae (Fig. 7b). Dorsal pilaster indistinct from the columns of wall pustules, and consisting of 5 broad, nested A-shapes. Basal one-third of the penis smooth with random folds; upper two-thirds sculpted with slightly separated columns of cuboidal, rough-surfaced pustules, enlarging and merging and ventrally into 6–10 tapered U-shapes. Sheath enclosing less than half of the upper, sculpted region of the penis.

Xolotrema fosteri (F. C. Baker, 1932)—Dissections: 6 from 2 populations. Fig. 8a. Length: 8 mm. Shape cylindrical. Ejaculatory pore terminal, on a verge. Verge small (0.2 mm), longer than wide, terminal, dorso-laterally compressed, backpointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 6 terminal papillae. Dorsal pilaster short (2 mm), moderately wide (mid-width 0.3 mm), and superficially resembling a single column of abutting cubes. Ventral surface bearing a long, smooth-surfaced, fleshy column with a central, longitudinal, shallow groove. Basal third of the penis smooth with random folds; middle third slightly bulbous and corrugated by bands of circular and longitudinal muscles; upper third sculpted with slightly separated columns of cuboidal, smooth-surfaced pustules, enlarging and merging and ventrally into 6–10 tapered U-shapes. Sheath enclosing the entire upper, sculpted region of the penis.

Xolotrema obstricta (Say, 1821)—Dissections: 2 from 1 population. Fig. 7c–d. Length: 11 mm. Shaped like an inverted pear.

Ejaculatory pore ventrally subterminal, about one-third-way from the penial apex in the upper, sculpted region, and on a verge. Verge small (0.2 mm), wider than long, ventrally subterminal on a slight prominence, dorsolaterally compressed, forward-pointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 4 narrow terminal papillae (Fig. 7d). Dorsal pilaster indistinct from the columns of wall pustules, and consisting of 5 broad, nested A-shapes. Basal one-third of the penis smooth with random folds; upper two-thirds sculpted with slightly separated columns of cuboidal, rough-surfaced pustules, enlarging and merging and ventrally into 6–10 tapered U-shapes. Sheath enclosing less than half of the upper, sculpted region of the penis.

Xolotrema occidentalis (Pilsbry & Ferriss, 1907)—Dissections: 1 from 1 population. Fig. 8b–c. Length: 7 mm. Shape cylindrical (in Fig. 8b, it appears clubbed because of contraction within the sheath). Ejaculatory pore terminal, on a verge. Verge small (0.3 mm), longer than wide, terminal, dorsolaterally compressed, back-pointing, with a ventrally subterminal pore and sculpted with surface cords continuing into about 6 terminal papillae (Fig. 8c). Dorsal pilaster short (1 mm), moderately wide (mid-width 0.3 mm), and superficially resembling a single column of abutting cubes. Ventral surface bearing a long, smooth-surfaced, fleshy column with a central, longitudinal, shallow groove (this structure is contracted and distorted in Fig. 8b). Basal third of the penis smooth with random folds; middle third slightly bulbous and corrugated by bands of circular and longitudinal muscles; upper third sculpted with slightly separated columns of cuboidal, smooth-surfaced pustules, enlarging and merging and ventrally into 6–10 tapered U-shapes. Sheath enclosing the entire upper, sculpted region of the penis.

Suggested character-state transformations

The total variation in penial morphology was classified into 10 characters comprising 60 character states. These are arranged into their suggested phylogenies in Figs. 19–23, in which the suggested character-state transformations are numbered 1–50.

The dorsal pilaster (Character 1) was the most variable penial-morphological character. Twenty-two states (including its absence in the outgroups) were detected, none of which appeared to be convergent. Their suggested phylogeny (Fig. 19) contains transformations 1–21.

Pustules on the penial wall (Character 2) yielded 11 character-states, with two sets of convergences, each involving three character-states: types 1, 2, and 3 chevrons; and 3 types of smooth columns (explained below). The suggested phylogeny (Fig. 20) involves transformations 22–33.

Verges (Character 3) occur in several of the outgroups of eastern triodopsines: *Vespericola,* Oreohelicidae, and some Camaenidae. These verges, because of their structural differences (discussed below), presumably are convergent on, rather than plesiomorphous with, the eastern-triodopsine verge. Six character states were detected, three of which appeared convergent (types 1, 2, and 3 small verges). The suggested character-state phylogeny (Fig. 21) embodies transformations 34-38.

The position on the penis of the ejaculatory pore, or opening of the vas deferens (Character 4), varied as 6 distinct character states, 4 of which appeared convergent (dorsally subterminal, and types 1, 2, and 3 ventrally subterminal). Evolution of a ventrally subterminal pore is probably easily achieved developmentally by overgrowth of the dorsal penial wall. This presumably is functionally adaptive because it both plugs the mate's spermathecal duct during copulation (with the overgrown apical knob of the penis) and emits the sperm mass beneath the plug and away from the digestive spermathecal bursa (Emberton, 1986). The convergences were detected by differences in penial shape and in details of pore position and structure, as explained below. The suggested character-state phylogeny (Fig. 22) contains transformations 39–43.

Characters 5–10 (ventral pilaster, basal penis length, ventral sperm channel, sheath length, upper penis length, and peduncle) each had two or three character states, suggested to be linked by one or two transformations (Fig. 23, transformations 44–50).

In presenting each of the 50 suggested character-state transformations below, the same format has been used throughout: (1) the transformation's identification number as used in Figs. 19–23; (2) the identification numbers of the transformation or series of transformations suggested to have preceded it evolutionarily; (3) the suggested plesio-

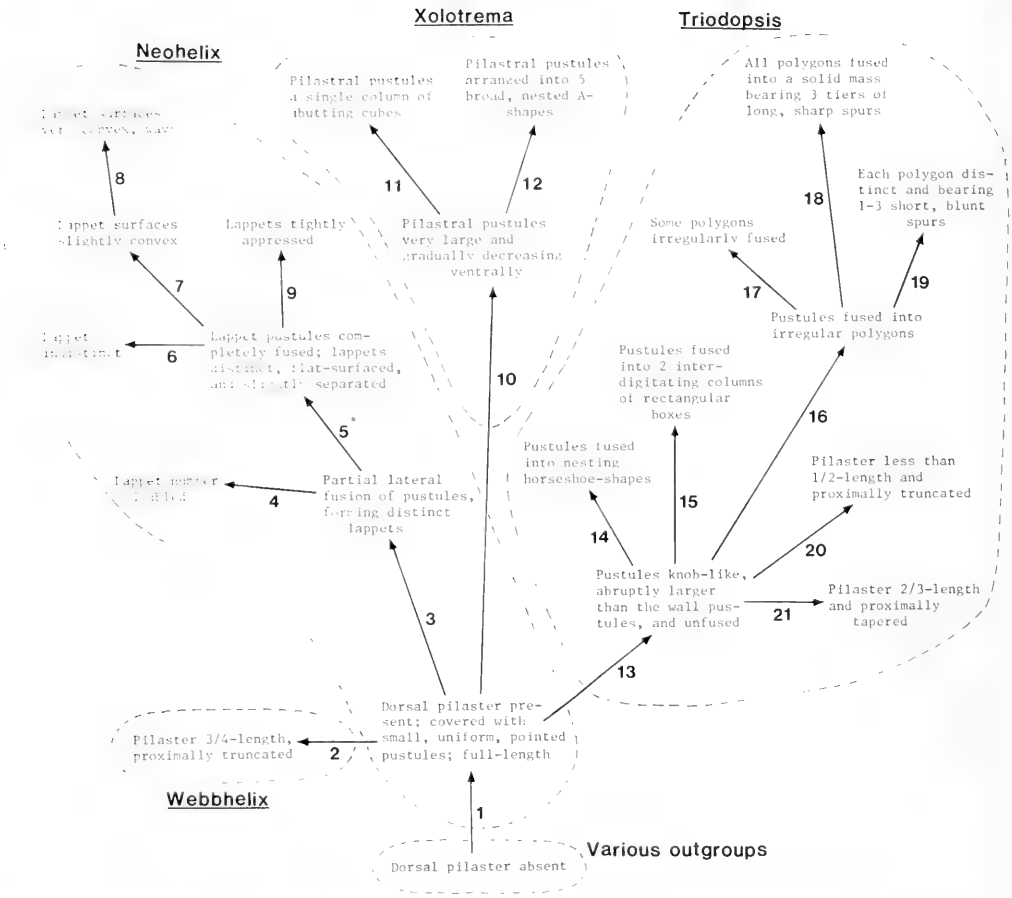

FIG. 19. Suggested character-state transformations in eastern American triodopsine penial morphology. Character 1, pilaster and pilastral pustules.

morphous state; (4) the outgroup taxa possessing the suggested plesiomorphous state; (5) the suggested apomorphous state; (6) the taxa suggested to have formerly possessed the apomorphous state, *although lacking it now;* (7) the taxa which now possess the suggested apomorphous state; and (8) a discussion of the suggested transformation, including its further explanation, if necessary, and its justification.

Transformation 1—Preceding transformations: none.

Plesiomorphous state: dorsal pilaster absent. Present in: *Cryptomastix, Allogona, Ashmunella, Oreohelix, Polygyrella,* Polygyracea, and the camaenids *Amplirhagada* and *Torresitrachea.*

Apomorphous state: dorsal pilaster present, covered with unmodified pustules, and full-length. Formerly present in: all eastern triodopsines (Figs. 2–18). Now present in: *Webbhelix multilineata* (Fig. 6a).

Discussion. Emberton (1986) discussed the evidence that this type of single dorsal pilaster, formed by a longitudinal outgrowth from the penial wall, is unique to triodopsines within the Polygyridae and their outgroups (a similar pilaster in some Australian camaenids is considered convergent). It apparently occurs in all eastern triodopsines (Figs. 2–18); although it is not at all obvious in the dissections of *Xolotrema* (Figs. 7, 8), it shows up clearly in cross-sections of the penes of *X. denotata* and *X. fosteri* (Pilsbry 1940, fig. 473 #6b, 7b: 793), so presumably occurs

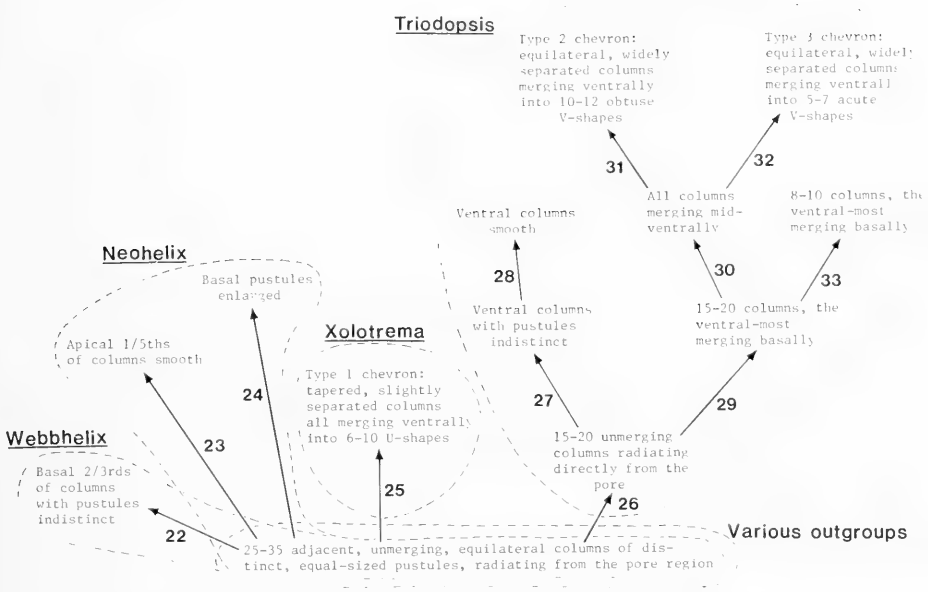

FIG. 20. Suggested character-state transformations in eastern American triodopsine penial morphology. Character 2, wall pustules.

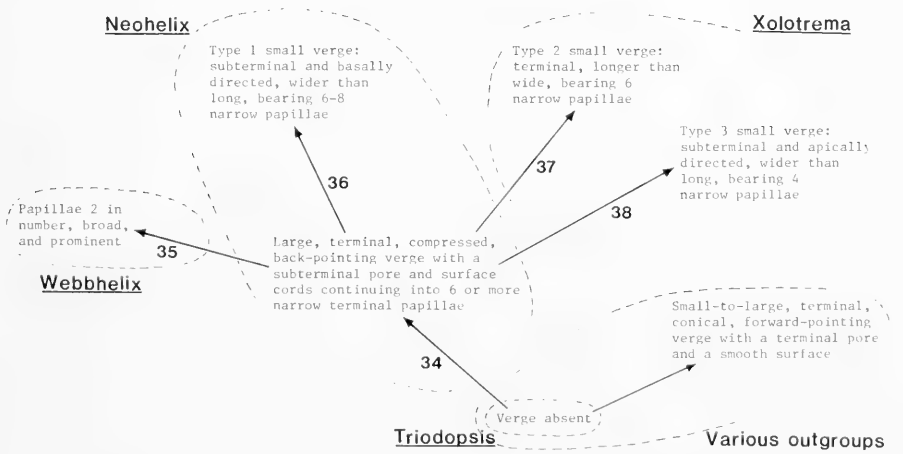

FIG. 21. Suggested character-state transformations in eastern American triodopsine penial morphology. Character 3, verge.

throughout the genus. It is assumed, for want of evidence to the contrary and because a similar structure appears nowhere else among the Polygyridae or their outgroups, that this single dorsal pilaster arose only once, so is homologous throughout eastern triodopsines. Its great variation in gross appearance appears to be attributable to differences in the patterns of fusion and enlargement of the pilastral pustules.

The most plesiomorphous pilastral sculpture appears to be that seen in *Webbhelix multilineata* (Fig. 6a). This pilastral sculpture is identical with the plesiomorphous wall

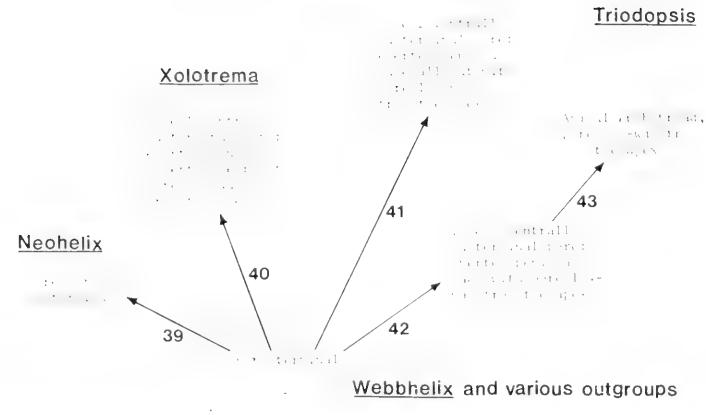

FIG. 22. Suggested character-state transformations in eastern American triodopsine penial morphology. Character 4, pore position.

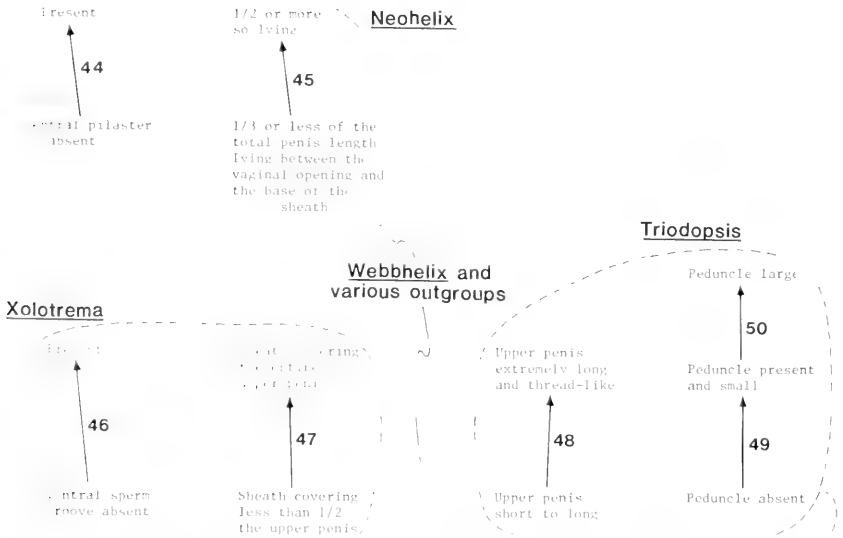

FIG. 23. Suggested character-state transformations in eastern American triodopsine penial morphology. Characters 5–10, ventral pilaster, basal penis length, ventral sperm groove, sheath length, upper penis length, and peduncle.

sculpture: adjacent, longitudinal columns of equal-sized pustules.

Transformation 2—Preceding transformations: 1.

Plesiomorphous state: pilaster full-length. Present in (outgroups): *Neohelix* (Figs. 2–5, 6b); *Xolotrema* (Figs. 7, 8); and *Triodopsis vulgata, picea, claibornensis* (Fig. 9), *burchi* (Fig. 11a), and *platysayoides* (Fig. 12).

Apomorphous state: pilaster 3/4-length and proximally truncated. Formerly and now present in: *Webbhelix multilineata* (Fig. 6a).

Discussion: This character state is unique to *W. multilineata,* so presumably is apomorphous.

Transformation 3—Preceding transformations: 1.

Plesiomorphous state: pilaster covered with unmodified pustules. Present in (outgroup): *Webbhelix multilineata* (Fig. 6a).

Apomorphous state: pilastral pustules partially fused laterally to form lappets. Formerly present in: all *Neohelix* (Figs. 2–5, 6b). Now present in: *Neohelix dentifera* (Fig. 2a, b), *lioderma* (Fig. 5a, c), and *divesta* (Fig. 5d, f).

Discussion. There appears to be a continuum from the totally unfused pilastral pustules of *W. multilineata* (Fig. 6a), to the laterally appressed slightly fused pilastral pustules of *N. dentifera* (Fig. 2b), to the partially laterally fused pilastral pustules of *N. lioderma* (Fig. 5c) and *N. divesta* (Fig. 5f), and the assumption is that this represents a true transformation series. This lateral fusion of pilastral pustules results in a column of overlapping, plate-like elements called "lappets" (Figs. 1, 2a, 5a, d).

Transformation 4—Preceding transformations: 1, 3.

Plesiomorphous state: lappets approximately equal in number to the number of columns of wall pustules. Present in (outgroup): *Neohelix albolabris* (Fig. 2d), *alleni* (Fig. 3a, c), *major* (Fig. 4a), and *solemi* (Fig. 6b).

Apomorphous state: lappet number doubled. Formerly and now present in: *Neohelix dentifera* (Fig. 2a), *lioderma* (Fig. 5a), and *divesta* (Fig. 5d).

Discussion. There are two distinct types of lappetted dorsal pilaster. In the first type, the number of pilastral lappets is approximately equal to (or somewhat greater than) the number of columns of wall pustules, as seen in *albolabris, alleni, major,* and *solemi.* In the other type, the number of pilastral lappets is approximately equal to twice the number of columns of wall pustules, as seen in *dentifera, lioderma,* and *divesta.* There are no intermediates between these two types. It appears likely that the double-lappet sculpture is derived from the single-lappet sculpture, possibly via a simple, one-step modification in a developmental program.

Transformation 5—Preceding transformations: 1, 3.

Plesiomorphous state: lappet pustules partially fused. Present in (outgroups): *Neohelix dentifera* (Figs. 2a, b), *lioderma* (Fig. 5a, c), and *divesta* (Fig. 5d, f).

Apomorphous state: lappet pustules completely fused. Formerly and now present in: *Neohelix albolabris* (Fig. 2d, e), *alleni* (Fig. 3a, c), *major* (Fig. 4a), and *solemi* (Fig. 6b).

Discussion. Although the lappets of *alleni,* and *major,* and *solemi* appear to have lost all trace of the pustules from which they presumably originated by lateral fusion, those of *albolabris* and *solemi* show a regular pattern of indentations (Figs. 2e and 6b) which seem to correspond to pustules (compare with Fig. 5c, f).

Transformation 6—Preceding transformations: 1, 3, 5.

Plesiomorphous state: lappets distinct. Present in (outgroups): *Neohelix dentifera, albolabris, alleni, major, lioderma,* and *divesta* (Figs. 2–5).

Apomorphous state: lappets indistinct. Formerly and now present in: *Neohelix solemi* (Fig. 6b).

Discussion: The dorsal pilaster of *solemi,* which appears on the right in Fig. 6b and is not to be confused with the ventral pilaster (unique to *solemi*) which appears in the center in Fig. 6b, is reduced in size and length due to the uniquely dorsally subterminal pore position (Transformation 38). Its lappets are indistinct and unequal in size, and may be vestigial now that a ventral pilaster is present.

Transformation 7—Preceding transformations: 1, 3, 5.

Plesiomorphous state: lappets flat-surfaced. Present in (outgroups): *Neohelix dentifera* (Fig. 2a), *alleni* (Fig. 3c), *lioderma* (Fig. 5a), and *divesta* (Fig. 5d).

Apomorphous state: lappets slightly convexly surfaced. Formerly present in: *albolabris* (Fig. 2d, e) and *major* (Fig. 4a). Now present in: *albolabris* (Fig. 2d, e).

Discussion. The convexity of *albolabris's* pilastral lappets (Fig. 2e) seems to result from a trend toward enlargement of the lappets which is continued in Transformation 8.

Transformation 8—Preceding transformations: 1, 3, 5, 7.

Plesiomorphous state: lappets slightly convexly surfaced. Present in (outgroup): *Neohelix albolabris* (Fig. 2d, e).

Apomorphous state: lappet surfaces very convex and irregularly wavy. Formerly and now present in: *Neohelix major* (Fig. 4a).

Discussion. This character state is unique

to *major,* which has the largest pilastral lappets, so presumably is apomorphous.

Transformation 9—Preceding transformations: 1, 3, 5.

Plesiomorphous state: lappets slightly separated. Present in (outgroups): *Neohelix albolabris* (Fig. 2a, e), *major* (Fig. 4a), and *solemi* (Fig. 6b).

Apomorphous state: lappets tightly appressed. Formerly and now present in: *Neohelix alleni* (Fig. 3c).

Discussion: The lappets are extremely smooth-surfaced and fit tightly together so that the general pilastral surface is relatively flat (Fig. 3c). Fig. 3a was accidently incorrectly shaded and does not properly represent this character state.

Transformation 10—Preceding transformations: 1.

Plesiomorphous state: pilastral pustules small and uniform in size. Present in (outgroup): *Webbhelix multilineata* (Fig. 6a).

Apomorphous state: pilastral pustules very large and gradually decreasing in size ventrally. Formerly and now present in: *Xolotrema* (Figs. 7a, c, e; 8a, b).

Discussion. *Xolotrema's* dorsal pilaster is unique and quite disjunct from any other found in eastern triodopsines. Its derivation from the *W. multilineata*-type is a best guess which is supported by the homologous verge (Character 3) between *Webbhelix* and *Xolotrema.* Whether the large pilastral pustules originated by enlargement, or fusion, or both, is beyond conjecture at this point.

Transformation 11—Preceding transformations: 1, 10.

Plesiomorphous state: pilastral pustules very large and gradually decreasing in size ventrally, but of unknown mid-dorsal configuration. Formerly present in: hypothesized common ancestor of *Xolotrema denotata, obstricta, caroliniensis, fosteri,* and *occidentalis* (Figs. 7, 8). Now present in: none.

Apomorphous state: pilastral pustules a single column of abutting cubes. Formerly and now present in: *Xolotrema fosteri* (Fig. 8a) and *occidentalis* (Fig. 8b).

Discussion. The superficial resemblance of *fosteri's* and *occidentalis's* pilastral elements (Fig. 8a, b) to lappets (e.g., Figs. 2d, 3a) and to polygons (e.g. Figs. 14a, 15a) breaks down on close examination; these three types appear to be nonhomologous. The dorsal pilaster of *fosteri* and *occidentalis* should not be confused with the ventral sperm groove (Figs. 8a, b) which occurs in these two species.

Transformation 12—Preceding transformations: 1, 10.

Plesiomorphous state: pilastral pustules very large and gradually decreasing in size ventrally, but of unknown mid-dorsal configuration. Present in: hypothesized common ancestor of *Xolotrema denotata, obstricta, caroliniensis, fosteri,* and *occidentalis* (Figs. 7, 8).

Apomorphous state: pilastral pustules arranged into 5 broad, nested A-shapes. Formerly and now present in: *Xolotrema denotata* (Fig. 7a), *obstricta* (Fig. 7c), and *caroliniensis* (Fig. 7e).

Discussion. The dorsal pilaster in *denotata, obstricta,* and *caroliniensis* is less obvious than in any other eastern triodopsines. The thickening of the dorsal penial wall which forms the dorsal pilaster is reduced in these species (Pilsbry 1940, fig. 473 #6b) to the extent that it is not at all evident in Fig. 7a, c, e. The swollen area beneath the subterminal verge in these species is easily mistaken for the dorsal pilaster, but the fact that the verge points up indicates that this is actually the ventral side of the penis, so the dorsal pilaster is on the opposite side (Fig. 7a, c, e), which is heavily sculpted with A-shaped arrangements of broad, rugose pustules. The substructural complexity of these pustules suggests that they resulted from fusion of smaller, plesiomorphous pustules. Despite the great difference between the dorsal pilastral sculptures of *denotata, obstricta,* and *caroliniensis* on one the hand (Fig. 7), and *fosteri* and *occidentalis* on the other hand (Fig. 8), their similarity in the broad, flat-surfaced, ventrally-diminishing pilastral pustules, as well as their apparent homologies in wall pustules (Character 2) and verge (Character 3), lead to the suggestion that their dorsal pilasters arose from a common, unknown ancestral type.

Transformation 13—Preceding transformation: 1.

Plesiomorphous state: pilastral pustules pointed and uniformly equal in size to the wall pustules. Present in (outgroup): *Webbhelix multilineata* (Fig. 6a).

Apomorphous state: pilastral pustules knob-like and abruptly larger than the wall pustules. Formerly present in: all *Triodopsis*

(Figs. 9–18). Now present in: *T. vulgata* (Fig. 9a) and *T. claibornensis* (Fig. 9c).

Discussion. Knob-like pilastral pustules about twice as large as the wall pustules, with no ventral intergradation in size, occur either unfused or fused in various ways in *vulgata, picea, claibornensis* (Fig. 9), *burchi* (Fig. 11a), and *platysayoides* (Fig. 12). The most similar pilastral sculpture, and one from which this type could easily have evolved, is that of *W. multilineata* (Fig. 6a), in which the pilastral pustules are more pointed and scale-like, and equal in size to the wall pustules: simple enlargement would have sufficed.

Transformation 14—Preceding transformations: 1, 13.

Plesiomorphous state: knob-like pilastral pustules unfused. Present in (outgroup): *Triodopsis vulgata* (Fig. 9a) and *claibornensis* (Fig. 9c).

Apomorphous state: pilaster sculpted with nesting horseshoe-shaped elements with a knobby surface. Formerly and now present in: *Triodopsis picea* (Fig. 9b).

Discussion. Despite their apparent fusion into this pattern, the apical knobs of the pilastral pustules are readily apparent in *picea.*

Transformation 15—Preceding transformations: 1, 13.

Plesiomorphous state: knob-like pilastral pustules unfused. Present in (outgroup): *Triodopsis vulgata* (Fig. 9a) and *claibornensis* (Fig. 9c).

Apomorphous state: pilaster sculpted with rectangular box-like elements with knobby surfaces and arranged in two interdigitating columns. Formerly and now present in *Triodopsis platysayoides* (Fig. 12).

Discussion. The knobby surface of *playtsayoides's* pilaster suggests that its box-like elements derived by fusion from the knob-like pilastral pustules seen in *vulgata* and *claibornensis.*

Transformation 16—Preceding transformations: 1, 13.

Plesiomorphous state: knob-like pilastral pustules unfused. Present in (outgroup): *Triodopsis vulgata* (Fig. 9a) and *claibornensis* (Fig. 9c).

Apomorphous state: pilaster sculpted with elements (polygons) 4–10 times the size of wall pustules and bearing 2–5 knobs. Formerly present in: hypothesized ancestor of all of *Triodopsis* except *vulgata, picea, claibornensis,* and *platysayoides* (Figs. 10, 11, 13–18). Now present in: none.

Discussion. These polygonal pilastral elements, which occur with modification throughout most of *Triodopsis,* have a surface sculpture or substructure reminiscent of the unfused pilastral pustules of *vulgata* and *claibornensis* (Fig. 9a, c)—this is especially evident in the illustration of *messana* (Fig. 16b)—so the assumption is that they were derived from these by fusion.

Transformation 17—Preceding transformations: 1, 13, 16?

Plesiomorphous state (?): knobby-surfaced pilastral polygons. Present in (outgroup): hypothesized ancestor approximated by the illustration of *Triodopsis messana* (Fig. 16b).

Apomorphous state: knobby-surfaced pilastral elements 1–2 times as large as polygons. Formerly and now present in: *Triodopsis burchi* (Fig. 11a).

Discussion. The pilastral sculpture of *burchi* is unique and problematic. Because of its knobby surface, it probably derived from either a *vulgata*-type ancestor (Fig. 9a) or a hypothesized ancestor in which partial fusion (into polygons) of the pilastral pustules had already taken place. The latter alternative was chosen because the irregular size and pattern of *burchi's* pilastral elements suggest that intermediate fusion has taken taken place.

Transformation 18—Preceding transformations: 1, 13, 16?

Plesiomorphous state (?): knobby-surfaced pilastral polygons. Present in (outgroup): hypothesized ancestor approximated by the illustration of *Triodopsis messana* (Fig. 16b).

Apomorphous state: dorsal pilaster a solid, rounded mass bearing about 3 tiers of long, sharp spurs. Formerly and now present in: *Triodopsis tennesseensis* (Fig. 11b) and *complanata* (Fig. 11d).

Discussion. This is another unique and problematic form of the dorsal pilaster. The spurs are so much longer, sharper, and more regularly arranged than are the blunt spurs of Transformation 19 that they are probably not homologous. The substructure of *complanata's* pilaster (Fig. 11d) somewhat resembles a hypertrophied and regularized form of, for example, the pilaster of *tridentata* (Fig. 14a), so it may have derived from a pilaster with polygonal elements.

Transformation 19—Preceding transformations: 1, 13, 16.

Plesiomorphous state: pilastral polygons with simple knobby surface. Present in (outgroup): hypothesized ancestor most closely approximated by the illustration of *Triodopsis messana* (Fig. 16b).

Apomorphous state: pilastral polygons bearing blunt spurs. Formerly and now present in: *Triodopsis fraudulenta* (Fig. 10), *vultuosa, cragini, henriettae, tridentata, anteridon, juxtidens, discoidea, hopetonensis, palustris, obsoleta, alabamensis, messana, vannostrandi* (Figs. 13–16); *fallax* (Fig. 17a), *neglecta, fulciden,* and *pendula* (Fig. 18).

Discussion. The blunt spurs, which are most obvious in the illustrations of *vultuosa* (Fig. 13a), *tridentata* (Fig. 14a), *anteridon* (Fig. 14b), and *alabamensis* (Fig. 16a), appear to be derived by outgrowth of the individual pustules which originally fused (Transformation 16) to form the polygons.

Transformation 20—Preceding transformations: 1.

Plesiomorphous state: dorsal pilaster full-length. Present in (outgroups): *Webbhelix* (Fig. 6a), *Neohelix* (Figs. 2–5, 6b);*Xolotrema* (Figs. 7, 8); and *Triodopsis vulgata, picea, claibornensis* (Fig. 9), *burchi* (Fig. 11a), and *platysayoides* (Fig. 12).

Apomorphous state: dorsal pilaster less than 1/2 length and proximally truncated. Formerly and now present in: *Triodopsis tennesseensis* (Fig. 11b) and *complanata* (Fig. 11d).

Discussion. This short pilaster appears apomorphous relative to the taxonomically widespread and probably ontogenetically more easily achieveable full-length pilaster.

Transformation 21—Preceding transformations: 1.

Plesiomorphous state: dorsal pilaster full-length. Present in (outgroups): *Webbhelix* (Fig. 6a), *Neohelix* (Figs. 2–5, 6b); *Xolotrema* (Figs. 7, 8); and *Triodopsis vulgata, picea, claibornensis* (Fig. 9), *burchi* (Fig. 11a), and *platysayoides* (Fig. 12).

Apomorphous state: dorsal pilaster 2/3 length and proximally tapered. Formerly and now present in: *Triodopsis fraudulenta* (Fig. 10), *vultuosa, cragini, henriettae, tridentata, anteridon, juxtidens, discoidea, hopetonensis, palustris, obsoleta, alabamensis, messana, vannostrandi* (Figs. 13–16), *fallax* (Fig. 17a), *neglecta, fulciden,* and *pendula* (Fig. 18).

Discussion. For the same reasons cited for Transformation 20, it is assumed that this shortened pilaster is apomorphous.

Transformation 22—Preceding transformations: none.

Plesiomorphous state: all wall columns bearing distinct pustules along their entire lengths. Present in (outgroups): some Camaenidae, Oreohelicidae, and Ashmunellinae; all *Neohelix* except *alleni* (Figs. 2, 4, 5, 6b).

Apomorphous state: all wall columns with pustules indistinct basally. Formerly and now present in: *Webbhelix multilineata* (Fig. 6a).

Discussion. Despite their partial fusion in *multilineata,* the wall pustules are still evident and give the penial wall a rough surface sculpture. Thus this character state differs from the smooth pustular columns discussed under Transformations 23, 27, and 28.

Transformation 23—Preceding transformations: none.

Plesiomorphous state: all wall columns bearing distinct pustules along their entire lengths. Present in (outgroups): some Camaenidae, Oreohelicidae, and Ashmunellinae; all *Neohelix* except *alleni* (Figs. 2, 3, 5, 6b).

Apomorphous state: all wall columns with their apical 1/5th to 1/4th smooth, with no trace of pustules. Formerly and now present in: *N. alleni* (Fig. 3).

Discussion. Although smooth wall columns occur in some other eastern triodopsines (Transformations 27 and 28), the apical localization seen in *alleni* is unique and surely nonhomologous.

Transformation 24—Preceding transformations: none.

Plesiomorphous state: all wall pustules approximately equal in size or smaller basally. Present in (outgroups): some Camaenidae, Oreohelicidae, and Ashmunellinae; all *Neohelix* except *dentifera, lioderma,* and *divesta* (Figs. 2d, 3, 4, 6).

Apomorphous state: basal wall pustules more than twice as large as the apical wall pustules. Formerly and now present in: *Neohelix dentifera* (Fig. 2a), *lioderma* (Fig. 5a), and *divesta* (Fig. 5d).

Discussion. There is variation within the apomorphous state: in *dentifera* the basal enlargement is more localized and abrupt than in *lioderma* and *divesta.* Because it is

unclear which of these variations would be plesiomorphous to the other, they were left together as one apparently homologous, apomorphous state.

Transformation 25—Preceding transformations: none.

Plesiomorphous state: wall columns unmerging, 25–35 in number, linear, equilateral, adjacent, and bearing equal sized-pustules. Present in (outgroups): some Camaenidae, Oreohelicidae, and Ashmunellinae; *Webbhelix;* all *Neohelix* except *dentifera, lioderma,* and *divesta* (Figs. 2d, 3, 4, 6).

Apomorphous state: Type 1 chevron: wall columns all merging mid-ventrally into 6–10 U-shapes, tapered, slightly separated, and bearing unequally sized pustules. Formerly and now present in: *Xolotrema* (Figs. 7, 8).

Discussion. This and similar patterns are being called "chevrons" because the ventral wall resembles an inverted chevron. Despite a superficial similarity to Types 2 and 3 chevrons (Transformations 31 and 32), the Type 1 chevron can be recognized as convergent by its ventral U- rather than V-shapes, its tapered rather than equilateral columns, its slightly rather than widely separated columns, its unequally rather than equally sized pustules, and its flat-surfaced rather than pointed pustules. The gap between the Type 1 chevron and its hypothesized plesiomorphous state is great and there are no other character states that appear transitional. The series represented by Transformations 29, 30, and 31 or 32 (discussed below) outlines a possible path similar to one by which the Type 1 chevron may have arisen independently.

Transformation 26—Preceding transformations: none.

Plesiomorphous state: 25–35 columns radiating from the pore region and adjacent and equally sized along their entire lengths. Present in (outgroups): some Camaenidae, Oreohelicidae, and Ashmunellinae; *Webbhelix;* and *Neohelix* (Figs. 2–6).

Apomorphous state: 15–20 columns radiating directly from and diverging and/or enlarging from the pore. Formerly present in: *Triodopsis* (Figs. 9–18). Now present in *Triodopsis vulgata, picea,* and *claibornensis* (Fig. 9).

Discussion. This wall-pustular pattern, in combination with a subterminal pore (Transformation 41), takes on the distinctive appearance of a spider's orb-web (especially Fig. 9a). It is closest to the presumably plesiomorphous pattern seen in the triodopsine outgroups and in *Webbhelix* and *Neohelix,* and could have arisen by fusion and/or simple reduction of wall columns.

Transformation 27—Preceding transformations: 26.

Plesiomorphous state: 15–20 radiating wall columns, all with distinct pustules. Present in (outgroup): *Triodopsis vulgata, picea,* and *claibornensis* (Fig. 9).

Apomorphous state: 15–20 radiating wall columns, the ventral-most with indistinct pustules. Formerly present in *Triodopsis fraudulenta, burchi, tennesseensis, complanata,* and *fulciden* (Figs. 10, 11, 18b). Now present in: *Triosopsis fraudulenta, rugosa* (Fig. 10), *burchi* (Fig. 11a), and *fulciden* (Fig. 18b).

Discussion. The ventral wall columns are semi-smooth, apparently due to either the partial fusion or partial loss of their pustules. Similarity to the indistinct wall pustules of *Webbhelix multilineata* (Fig. 6a) is due to convergence.

Transformation 28—Preceding transformations: 26, 27.

Plesiomorphous state: 15–20 radiating wall columns, the ventral-most with indistinct pustules. Present in (outgroup): *Triodopsis fraudulenta* (Fig. 10), *burchi* (Fig. 11a), and *fulciden* (Fig. 18b).

Apomorphous state: 15–20 radiating wall columns, the ventral-most smooth, with no trace of pustules. Formerly and now present in *Triodopsis tennesseensis* (Fig. 11b, c) and *complanata* (Fig. 11d).

Discussion. The assumption is that the ventral pustules of *tennesseensis* and *complanata* did not become smooth directly, but passed through a semi-smooth stage homologous with that of *fraudulenta, burchi,* and *fulciden.*

Transformation 29—Preceding transformations: 26.

Plesiomorphous state: 15–20 radiating wall columns, the ventral-most unmerging. Present in (outgroup): *Troiodopsis vulgata, picea, claibornensis* (Fig. 9), *burchi, tennesseensis,* and *complanata* (Fig. 11).

Apomorphous state: 15–20 radiating wall columns, the ventral-most merging basally. Formerly present in: all *Triodopsis* except *vulgata, picea, claibornensis, burchi, tennesseensis,* and *complanata* (Figs. 10, 12–18).

Now present in: *Triodopsis fraudulenta, rugosa* (Fig. 10), and *fulciden* (Fig. 18b).

Discussion. The ventral wall columns form spindle shapes by diverging from the pore, then merging basally. The basal merging presumably derived from non-merging columns, probably by a simple change in the developmental program by which the pustular columns form.

Transformation 30—Preceding transformations: 26, 29.

Plesiomorphous state: 15–20 wall columns, the ventral-most merging basally. Present in (outgroup): *Triodopsis fraudulenta, rugosa* (Fig. 10), and *fulciden* (Fig. 18b).

Apomorphous state: all 15–20 wall columns merging midventrally to form a plesiomorphous inverted chevron pattern. Formerly present in: *Triodopsis platysayoides, vultuosa, cragini, henriettae, tridentata, anteridon, juxtidens, discoidea, hopetonensis, palustris, obsoleta, alabamensis, messana, vannostrandi, fallax, soelneri, neglecta,* and *pendula* (Figs. 12–18). Now present in: none.

Discussion: The ventral wall patterns of *platysayoides* (Type 2 chevron) and the remaining species (Type 3 chevron) differ significantly, but have enough features in common that they probably had a common ancestor of unknown appearance, but probably closer to *platysayoides* because of the number of pustular columns involved. It is assumed that the mid-ventral merging of wall columns evolved in a basal-to-apical direction, with an intermediate stage in this process represented by the basal merging seen in *fraudulenta, rugosa,* and *fulciden.* Ontogenetic studies of penial sculpture may prove useful in testing this assumption.

Transformation 31—Preceding transformations: 26, 29, 30.

Plesiomorphous state: all 15–20 wall columns merging midventrally to form a plesiomorphous inverted chevron pattern. Present in (outgroup): hypothesized ancestor probably closest to *Triodopsis platysayoides* (Fig. 12).

Apomorphous state: Type 2 chevron: wall columns all merging midventrally into 10–12 obtuse V-shapes, equilateral, widely separated, and bearing equally sized pustules. Formerly and now present in *Triodopsis platysayoides* (Fig. 12).

Discussion. This Type 2 chevron is convergent on Types 1 and 3 chevrons. For differences from the Type 1 chevron, see the discussion under Transfomation 25. The Type 2 differs from the Type 3 chevron (Transformation 32) by the greater number and more obtuse angle of its ventral V-shapes.

Transformation 32—Preceding transformations: 26, 29, 30.

Plesiomorphous state: all 15–20 wall columns merging midventrally to form an inverted chevron pattern. Present in (outgroup): hypothesized ancestor probably closest to *Triodopsis platysayoides* (Fig. 12).

Apomorphous state: Type 3 chevron: wall columns all merging mid-ventrally into 5–7 acute V-shapes, equilateral, widely separated, and bearing equally sized pustules. Formerly and now present in: *Triodopsis vultuosa, cragini, henriettae, tridentata, anteridon, juxtidens, discoidea, hopetonensis, palustris, obsoleta, alabamensis, messana, vannostrandi, fallax, soelneri, neglecta,* and *pendula* (Figs. 12–18).

Discussion. There is considerable variation within this character state, and a more thorough and extensive study may break it down into a number of systematially useful categories. What is considered the "basic" Type 3 chevron is well represented in the illustrations of *hopetonensis* (Fig. 26a), *palustris* (Fig. 15b), and *alabamensis* (Fig. 16a); it differs from the Type 2 chevron Transformation 31) by the lesser number and the more acute angle of its ventral V-shapes; for its difference from the Type 1 chevron, see the discussion under Transformation 25. Variations from the "basic" Type 3 chevron include the more acutely angled V-shapes, presumably due to penial elongation, in *vultuosa, cragini,* and *henriettae* (Fig. 13); the apparent partial effacement of the wall sculpture of *juxtidens* (Fig. 14c), possibly due to sympatry with the similar *tridentata* (Fig. 14a); the possible effacement of the wall sculpture of *fallax* (Fig. 17a), which may be an artifact of the strong contraction of this specimen; the apparently total effacement of the wall sculpture of *soelneri* (Fig. 17b), possibly due to sympatry with the similar *hopetonensis* (Fig. 15a) and *messana* (Fig. 16b) (see Table 8); and the anastomoses among the V-shapes in *pendula* (Fig. 18c). Deriving both Types 2 and 3 chevrons from a common ancestor is a parsimonious suggestion, but need not be true: the apparent independent origin of the Type 1 chevron is evidence that Types 2 and

3 could have evolved independently of each other as well.

Transformation 33—Preceding transformations: 26, 29.

Plesiomorphous state: 15–20 radiating wall columns, the ventral-most merging basally. Present in (outgroup): *Triodopsis fraudulenta* and *rugosa* (Fig. 10).

Apomorphous state: 8–10 radiating wall columns, the ventral-most merging basally. Formerly and now present in: *Triodopsis fulciden* (Fig. 18b).

Discussion. The wall pattern of *fulciden* is unique and problematic. It most closely resembles *rugosa* (not illustrated, but similar to *fraudulenta* (Fig. 10)), except that the number of pustular columns is approximately halved. This reduction would parallel the reduction in column number suggested in Transformation 32.

Transformation 34—Preceding transformations: none.

Plesiomorphous state: pore flush with the penial wall. Present in (outgroups): some Camaenidae; and Ammonitellidae, *Allogona, Cryptomastix,* and *Triodopsis* (Figs. 9–18).

Apomorphous state: pore ventrally subterminal on a large, apical, dorsoventrally compressed verge which points backward along the everted penis, and with a surface sculpture of cords which continue into 6 or more narrow terminal papillae. Formerly present in: *Webbhelix* (Fig. 6a), *Neohelix* (Figs. 2–6), *Xolotrema* (Figs. 7, 8). Now present in: *Neohelix dentifera, albolabris, alleni, major, lioderma,* and *divesta* (Figs. 2–5).

Discussion. The hypothesized generalized form of the eastern-triodopsine verge is illustrated in dorsal view in Figs. 2a, d, 3a, c, 4a, 5d; and in magnified ventral view, showing the subterminal pore, in Figs. 2f, 3b, 4b, and 4e. The verge of the illustrated specimen of *lioderma* (Fig. 5a, b) is abnormally partially inverted; in undistorted specimens, this species's verge resembles that of *divesta* (Fig. 5d, e). Despite careful search (e.g., Fig. 11c), no trace of a verge was detected in any species of *Triodopsis*—the peduncle (Transformations 45 and 46), despite a superficial resemblance to a verge (e.g. Fig. 14a–d), differs in both position and structural detail. Although Webb's (1961, 1974) hypothesis that *Triodopsis* has secondarily lost the verge cannot be ruled out, this genus's complete lack of any vestige suggests rather that it never had a verge.

Transformation 35—Preceding transformations: 34.

Plesiomorphous state: vergic papillae 6 or more, narrow. Present in (outgroups): *Neohelix* (Figs. 2–5, 6b).

Apomorphous state: vergic papillae 2, broad. Formerly and now present in: *Webbhelix multilineata* (Fig. 6a).

Discussion. Narrow papillae appear to be simple extensions of the basic cord-like substructure of the verge (e.g. Fig. 2f). These cords and papillae are probably homologous with wall-pustular columns. It seems likely that the broad, paired papillae of *multilineata* are derived by fusion of the plesiomorphous narrow papillae. The surface of *multilineata's* verge also appears to be smooth, lacking the cord-like substructure (Fig. 6a).

Transformation 36—Preceding transformations: 34.

Plesiomorphous state: verge large, terminal, longer than wide, bearing 6 or more narrow papillae. Present in (outgroup): *Neohelix dentifera, albolabris, alleni, major, lioderma,* and *divesta* (Figs. 2–5).

Apomorphous state: Type 1 small verge: subterminal and basally-directed, wider than long, bearing 6–8 narrow papillae. Formerly and now present in: *Neohelix solemi* (Fig. 6b).

Discussion. Small size in the eastern-triodopsine verge is correlated with a subterminal position (Types 1 and 3) and with a long penis (slight in Type 3, pronounced in Type 2). Since both a subterminal pore (Character 4) and a long penis (Character 9) seem to be apomorphous conditions (see discussions under Transformations 38–41, 44), small size of the verge may also be apomorphous. In the case of subterminal pore position, this view is supported both by structural differences, indicating convergence, between dorsally subterminal (Type 1) and ventrally subterminal (Type 3) small verges; and by the following theory on functional morphology.

Because the terminal verge of eastern triodopsines unfolds backward during copulation (Webb 1948, 1952, 1954a; see Fig. 1), it is hypothesized that its function is to direct the emitted sperm backward and away from the proteolytic enzymes of the spermathecal bulb. This function seems also to be served, however, by the alternative adaptive "strat-

egy" of moving the pore from a terminal to a subterminal position. Therefore, when the pore becomes subterminal, the verge is no longer functional, and therefore becomes vestigial. This theory, however, does not explain the apparent correlation between a long penis and a short type-2 verge.

Transformation 37—Preceding transformations: 34.

Plesiomorphous state: verge large, terminal, longer than wide, bearing 6 or more narrow papillae. Present in (outgroup): *Neohelix dentifera, albolabris, alleni, major, lioderma,* and *divesta* (Figs. 2–5).

Apomorphous state: Type 2 small verge: terminal, longer than wide, bearing 6 narrow papillae. Formerly and now present in: *Xolotrema fosteri* and *occidentalis* (Fig. 8).

Discussion. Structurally this Type 2 small verge differs from the Type 3 (Transformation 38) only in being longer than wide and in having two more papillae (Fig. 8c vs. Fig. 7b, d), but further comparative study may prove this latter difference insignificant. There is the possibility, therefore, that Types 2 and 3 small verges are homologous, but because of their different positions (terminal vs. subterminal), it is suggested that they have independently become vestigial for functionally different reasons. By similar reasoning, Types 2 and 1 small verges are also convergent.

Transformation 38—Preceding transformations: 34.

Plesiomorphous state: verge large, terminal, longer than wide, bearing 6 or more narrow papillae. Present in (outgroup): *Neohelix dentifera, albolabris, alleni, major, lioderma,* and *divesta* (Figs. 2–5).

Apomorphous state: Type 3 small verge: subterminal and apically directed, wider than long, bearing 4 narrow papillae. Formerly and now present in: *Xolotrema denotata, obstricta,* and *caroliniensis* (Fig. 7).

Discussion. Structurally, this Type 3 small verge (Fig. 7b, d) differs from the Type 1 (Transformation 36) only in having two less papillae, but its vastly different position—ventrally subterminal as opposed to dorsally subterminal—leads to the suggestion that Types 1 and 3 are convergent. The suggested homoplasy Types 3 and 2 is discussed under Transformation 37.

Transformation 39—Preceding transformations: none.

Plesiomorphous state: pore terminal. Present in (outgroups): many Camaenidae; Corillidae, Ammonitellidae, Oreohelicidae; all non-east-American-triodopsine Polygyridae; *Webbhelix;* all *Neohelix* except *solemi; Xolotrema fosteri* and *occidentalis;* and *Triodopsis fraudulenta, burchi, tennesseensis, complanata, platysayoides, vultuosa, cragini, henriettae,* and *fulciden* (Figs. 2–5, 6a, 8, 10–13, 18b).

Apomorphous state: pore dorsally subterminal and on a fleshy pedestal. Formerly and now present in: *Nehoelix solemi* (Fig. 6b).

Discussion. The orientation of the verge and the position of the reduced dorsal pilaster clearly indicate the uniquely dorsal position of the pore in *solemi.*

Transformation 40—Preceding transformations: none.

Plesiomorphous state: pore terminal. Present in (outgroups): many Camaenacea; Corillidae, Ammonitellinidae, Oreohelicidae; all non-eastern-triodopsine Polygyridae; *Webbhelix;* all *Neohelix* except *solemi; Xolotrema fosteri* and *occidentalis;* and *Triodopsis fraudulenta, burchi, tennesseensis, complanata, platysayoides, vultuosa, cragini, henriettae,* and *fulciden* (Figs. 2–5, 6a, 8, 10–13, 18b).

Apomorphous state: Type 1 ventrally subterminal pore: everted penis shaped like an inverted pear, with the pore ca 1/3-way from the apex and on a verge mounted on a fleshy pedestal. Formerly and now present in: *Xolotrema denotata, obstricta,* and *caroliniensis* (Fig. 7).

Discussion. Webb published two illustrations of the everted penis of *denotata* (Webb 1948, Fig. 1a; Webb 1954a, Plate 10, Fig. 11), showing its pyrifom shape and its pore (verge) position. Fig. 7 shows that the dissected, uneverted penis also has this shape, that *obstricta* and *caroliniensis* are essentially identical to *denotata* in this respect, and that the pore is on a fleshy pedestal which is not evident in the everted penis.

Transformation 41—Preceding transformations: none.

Plesiomorphous state: pore terminal. Present in (outgroups): many Camaenidae; Corillidae, Ammonitellinidae, Oreohelicidae; all non-eastern-triodopsine Polygyridae; *Webbhelix;* all *Neohelix* except *solemi; Xolotrema fosteri* and *occidentalis;* and *Triodopsis*

fraudulenta, burchi, tennesseensis, complanata, platysayoides, vultuosa, cragini, henriettae, and *fulciden* (Figs. 2–5, 5a, 8, 10–13, 18b).

Apomorphous state: Type 2 ventrally subterminal pore: everted penis shaped like an angled baseball bat, with the pore ca 1/5-way from the apex and indented into the penial wall. Formerly and now present in: *Triodopsis vulgata, picea,* and *claibornensis* (Fig. 9).

Discussion. Five published illustrations of the everted penis of *vulgata* (Webb 1959, Figs. 22, 27, 34, 38) show the general shape and position of the pore. Fig. 9 (this paper) shows that the dissected, uneverted penis also has this general shape, that *picea* and *claibornensis* are essentially identical to *vulgata* in this respect, and that there is no sign of a pedestal or verge. Webb's figures indicate a sub-pore protuberance ("tubercle") which is covered with pustular columns (Webb 1959, Fig. 38); this is not evident in the uneverted penis (Fig. 9).

Transformation 42—Preceding transformations: none.

Plesiomorphous state: pore terminal. Present in (outgroups): many Camaenidae; Corillidae, Ammonitellinidae, Oreohelicidae; all non-eastern-triodopsine Polygyridae; *Webbhelix;* all *Neohelix* except *solemi; Xolotrema fosteri* and *occidentalis;* and *Triodopsis fraudulenta, burchi, tennesseensis, complanata, platysayoides, vultuosa, cragini, henriettae,* and *fulciden* (Figs. 2–5, 5a, 8, 10–13, 18b).

Apomorphous state: Type 3a ventrally subterminal pore: everted penis shaped like a thick-handled mace, with the pore ca 1/4-way from the apex and above a smooth peduncle. Formerly present in: *Triodopsis juxtidens, discoidea, neglecta,* and *pendula* (Figs. 14c, d; 18a, c). Now present in: *Triodopsis tridentata, anteridon, hopetonensis, palustris, obsoleta, alabamensis, messana, vannostrandi, fallax,* and *soelneri* (Figs. 14a–b, 15–17).

Discussion. Illustrations of the everted penis of four of the ten species with a Type 3a ventrally subterminal pore have been published: *fallax* (Grimm 1975, Fig. 3B), *hopetonensis* (Webb 1959, Figs. 9, 42), *tridentata* (Webb 1948, Fig. 4; Webb 1954a, Fig. 13; Webb 1959, Figs. 14, 28, 35, 40), and *vannostrandi* (Webb 1959, Figs. 17, 25a, 43). Figures 14a–b, 15, 16, and 17 (this paper) show that the dissected, uneverted penes of these four species also have this shape and that the remaining 6 species are essentially identical in this respect.

Transformation 43—Preceding transformations: 42.

Plesiomorphous state: Type 3a ventrally subterminal pore: everted penis shaped like a thick-handled mace, with the pore ca 1/4-way from the apex and above a smooth peduncle. Present in (outgroup): *Triodopsis tridentata, anteridon, hopetonensis, palustris, obsoleta, alabamensis, messana, vannostrandi, fallax,* and *soelneri* (Figs. 14a–b, 15–17).

Apomorphous state: Type 3b ventrally subterminal pore: mace head large, with the pore ca 2/5-way from the apex. Formerly and now present in: *Triodopsis juxtidens* (Fig. 14c), *discoidea* (Fig. 14d), *neglecta* (Fig. 18a), and *pendula* (Flg. 18c).

Discussion. Illustrations of the everted penis of three of these four species have been published: *discoidea* (Webb 1959, Fig. 13), *juxtidens* (Webb 1959, Figs. 15, 41), and *neglecta* (Webb 1959, Figs. 10, 11, 12). Figures 14c, d and 18a, c (this paper) show that in the dissected, uneverted penes of these three species, the Type 3b ventrally subterminal pore is more easily distinguished from the Type 3a by the size of the peduncle (see discussion under Transformation 50) than by the position of the pore, which varies with the state of contraction. Thus, although the pore position of *discoidea* (Fig. 14d) is as it appears in the everted penis, the pores of *juxtidens* (Fig. 14c) and *neglecta* (Fig. 18a) are much closer to the apex than they appear in the everted penes. The pore of *pendula* (Fig. 18c) is intermediate in position and its peduncle is large, so *pendula* is interpreted as having a Type 3b ventrally subterminal pore. Since the Type 3b has a very similar penial shape and sub-pore peduncle to the Type 3a, it is assumed to be homologous and derived from the Type 3a by further overgrowth of the dorsal penial wall, making the terminal knob larger and the pore more subterminal.

Transformation 44—Preceding transformations: none.

Plesiomorphous state: ventral wall free of any pilastral outgrowth. Present in (outgroups): some Camaenidae; Oreohelicidae, Ammonitellidae, and all Triodopsinae except *Neohelix solemi* (Fig. 49).

Apomorphous state: ventral wall bearing a

single pilaster. Formerly and now present in: *Neohelix solemi* (Fig. 6b).

Discussion. Because of its surface sculpture of close, uniform pustules, the ventral pilaster of *solemi* is convergent on the dorsal pilaster of *multilineata* (side-by-side comparison in Fig. 6). It almost certainly is apomorphous.

Transformation 45—Preceding transformations: none.

Plesiomorphous state: 1/3 or less of the total penis length lying between the vaginal opening and the base of the sheath. Present in (outgroups): all eastern Triodopsinae except *Neohelix solemi* (Figs. 2–5, 6a, 7–18).

Apomorphous state: 1/2 or more of the total penis length lying between the vaginal opening and the base of the sheath. Formerly and now present in: *Neohelix solemi* (Fig. 6b).

Discussion. A long basal penis occurs in the polygyrid ashmunellines *Cryptomastix, Allogona,* and *Ashmunella,* but it appears that this character state in *Neohelix solemi* is not homologous because of its absence in *solemi's* more immediate outgroup, the remaining species of *Neohelix.*

Transformation 46—Preceding transformations: none.

Plesiomorphous state: mid-ventral wall free of sperm groove. Present in (outgroup): all eastern triodopsines except *Xolotrema fosteri* and *occidentalis* (Figs. 2–7, 9–18).

Apomorphous state: mid-ventral sperm groove present. Formerly and now present in: *Xolotrema fosteri* and *occidentalis* (Fig. 19).

Discussion. The term "ventral sperm groove" refers to the smooth, mid-ventral, raised channel shown (somewhat exaggeratedly) in Fig. 8a, b, and shown in cross section in Pilsbry (1940, Fig. 473 #7b), even though its function is unknown. It is not conspicuous in any of Webb's illustrations of the everted penis of *fosteri* (Webb 1952, Figs. 6, 8, 10, 11; Webb 1954a, Fig. 12), but is pronounced enough in the dissected, uneverted penis to be mistaken for the dorsal pilaster—transverse folds across the sperm groove in a contracted specimen of *occidentalis* (Fig. 8b) even produce a superficial resemblance to pilastral lappets (e.g., Fig. 2d).

Transformation 47—Preceding transformations: none.

Plesiomorphous state: sheath covering 1/2 or less of the upper penis. Present in (outgroup): all eastern triodopsines except *Xolotrema fosteri* and *occidentalis* (Figs. 2–7, 9–18).

Apomorphous state: sheath covering the entire upper penis. Formerly and now present in: *Xolotrema fosteri* and *occidentalis* (Fig. 8).

Discussion. Penial sheath length varies a great deal depending on the preservational state of the individual snail, so no attempt was made to analyze interspecific differences, with the single exception of this distinct and obviously apomorphous character state. The apparently long sheath of the illustrated specimen of *Neohelix dentifera* (Fig. 2a) resulted from prolapse of the upper penis into the basal penis, evidenced by the pattern of folds at the upper-basal junction—in other dissected specimens of *dentifera* the sheath appeared relatively shorter. The illustrated penis of *X. fosteri* (Fig. 8a) appears to be normal in length, but that of *X. occidentalis* (Fig. 8b) is obviously contracted within its uncontracted sheath.

Transformation 48—Preceding transformations: none.

Plesiomorphous state: upper penis short to long. Present in (outgroup): all eastern triodopsines except *Triodopsis vultuosa, cragini,* and *henriettae* (Figs. 2–12, 14–18).

Apomorphous state: upper penis extremely long and thread-like. Formerly and now present in *Triodopsis vultuosa, cragini,* and *henriettae* (Fig. 13).

Discussion. The length of the upper, pustulated, penis is subject to some individual variation depending on preservational state. Because of this, and because of the high probability of convergence in such a developmentally plastic character as overall length, penial length was not considered for systematic analysis except in this extreme and obviously apomorphous case. The everted penis (illustrated in Webb 1959, Figs. 26b, 26c, 32; and Grimm 1975, Fig. 3c) is conspicuously long and narrow.

Transformation 49—Preceding transformations: none.

Plesiomorphous state: sub-pore region flat or gradually raised. Present in (outgroup): *Webbhelix; Neohelix; Xolotrema;* and *Triodopsis vulgata, picea, claibornensis, fraudulenta, burchi, tennesseensis, complanata, platysayoides, vultuosa, cragini, henriettae,* and *fulciden* (Flgs. 2–13, 18b).

Apomorphous state: sub-pore region erectile as a small, fleshy peduncle. Formerly

present in: *Triodopsis juxtidens, discoidea, neglecta,* and *pendula* (Figs. 14c, d; 18a, c). Now present in: *Triodopsis tridentata, anteridon, hopetonensis, palustris, obsoleta, alabamensis, messana, vannostrandi, fallax,* and *soelneri* (Flgs. 14a–b, 15–17).

Discussion. The peduncle was defined by Webb (1959) as the smooth, fleshy knob just beneath the subterminal pore in the everted penis of *hopetonensis, tridentata, vannostrandi, discoidea, juxtidens,* and *neglecta.* It occurs in two disjunct sizes: small and large. In neither of these is there any substructural detail suggesting homology with the verge, so independent derivation is assumed, with the large peduncle derived from the small peduncle. The sub-pore "tubercle" of *vulgata* (Webb 1959) is not smooth but pustulose and is not a distinct knob, so probably is not homologous with the peduncle. The peduncle appears to consist of erectile tissue which variously appears in the dissected, uneverted penis as a lobe (Fig. 14a, b, c, d), thickened region (Figs. 15a, c; 16b; 17a, b), or wrinkled sac (Figs. 15b; 16a, c; 18a, c) beneath the pore. The erect small peduncle is best illustrated in Webb 1948, Fig. 4 (as the "protuberance"), and in Webb 1959, Figs. 14, 25a, 40, and 43.

Transformation 50—Preceding transformations: 49.

Plesiomorphous state: peduncle small. Present in (outgroup): *Triodopsis tridentata, anteridon, hopetonensis, palustris, obsoleta, alabamensis, messana, vannostrandi, fallax,* and *soelneri* (Flgs. 14a–b, 15–17).

Apomorphous state: peduncle large. Formerly and now present in: *Triodopsis juxtidens, discoidea, neglecta,* and *pendula* (Figs. 14c, d; 18a, c).

Discussion. The erect large peduncle is best illustrated in Webb 1959, Figs. 12, 13, 15, and 41. The large peduncle can be distinguished from the small peduncle in the dissected, uneverted penis by its large size, whether it is inflated (Fig. 14c, d) or deflated (Fig. 18a, c).

Cladistic analysis

The presence or absence of each of the 50 suggested anatomical transformations in each species of eastern triodopsines is presented in Table 1.

To simplify cladistic analysis, genitalically identical species were pooled, reducing the number of operational taxa from 40 species to 18 groups (Table 1). Nine of these groups consisted of a single species *(W. multilineata, N. solemi, N. albolabris, N. major, N. alleni, T. picea, T. fulciden, T. burchi,* and *T. platysayoides).* Each of the remaining multispecies groups was temporarily named for one of its better-known species without regard for previously named supraspecific taxa. By far the largest of these (Table 1) was the *T. tridentata* group, comprising 10 species *(tridentata, anteridon, fallax, obsoleta, palustris, messana, soelneri, alabamensis, vannostrandi,* and *hopetonensis).* One of these, *soelneri,* was problematic in that it lacks wall pustules and its pilaster lacks surface sculpture (Fig. 17b). However, because of its Type 3a ventrally subterminal pore, its small peduncle, and the basic similarity of its pilaster to a *tridentata*-type without the conical processes, *soelneri* was considered a highly derived member of the *tridentata* group. The remaining 8 multi-species groups were nonproblematic. The *N. dentifera* group had three species *(dentifera, divesta,* and *lioderma);* the *X. fosteri* group had two species *(fosteri* and *occidentalis);* the *X. denotata* group had three species *(denotata, obstricta,* and *carolinienesis);* the *T. vulgata* group had two species *(vulgata* and *claibournensis);* the *T. fraudulenta* group had two species *(faudulenta* and *rugosa);* the *T. tennesseensis* group had two species *(tennesseensis* and *complanata);* the *T. cragini* group had three species *(cragini, vultuosa,* and *henriettae);* and the *T. juxtidens* group had four species *(juxtidens, discoidea, neglecta,* and *pendula).*

Cladistic analysis resulted in a single most parsimonious tree (Fig. 24) with convergences in three transformations and with no reversals (consistency index = 48.5/50 = .970). The three convergences were in transformations 27, 29, and 30. The convergence in 27 was biologically probable, because two easily identifiable convergences were already known for this character (Transformations 22 and 23). Convergences in 29 and 30 were also not unreasonable, as had been noted in the discussion of Transformation 31. The most parsimonious way to avoid these two homoplasies involved an alternative placement of *T. platysayoides.* This alternative produced a tree with an overall consistency index only slightly lower (48/50 = .960), but it invoked three reversals in the *T. platysayoides* lineage (Transformations 16, 19, and 21). This was clearly an inferior alternative to

TABLE 1. Presence (1) or absence (0) of 50 hypothesized character-state transformations in each of the 40 species of eastern American triodopsines. The 18 groups consist of species with identical presence/absence patterns.

Temporary species group	Species	Transformation number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
—	OUTGROUPS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
multilineata	*multilineata*	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
solemi	*solemi*	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0
albolabris	*albolabris*	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
major	*major*	1	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
alleni	*alleni*	1	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
dentifera	*dentifera*	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
dentifera	*divesta*	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
dentifera	*lioderma*	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fosteri	*fosteri*	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0
fosteri	*occidentalis*	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0
denotata	*denotata*	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
denotata	*obstricta*	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
denotata	*caroliniensis*	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
vulgata	*vulgata*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
vulgata	*clabornensis*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
picea	*picea*	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
fraudulenta	*fraudulenta*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fraudulenta	*rugosa*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fulciden	*fulciden*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
tennesseensis	*tennesseensis*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
tennesseensis	*complanata*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
burchi	*burchi*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
platysayoides	*platysayoides*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
cragini	*cragini*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
cragini	*vultuosa*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
cragini	*henriettae*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
tridentata	*tridentata*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
tridentata	*anteridon*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
tridentata	*fallax*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
tridentata	*messana*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
tridentata	*palustris*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
tridentata	*obsoleta*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
tridentata	*soelneri*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
tridentata	*alabamensis*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
tridentata	*vannostrandi*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
tridentata	*hopetonensis*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
juxtidens	*juxtidens*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1
juxtidens	*discoidea*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1
juxtidens	*neglecta*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1
juxtidens	*pendula*	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1

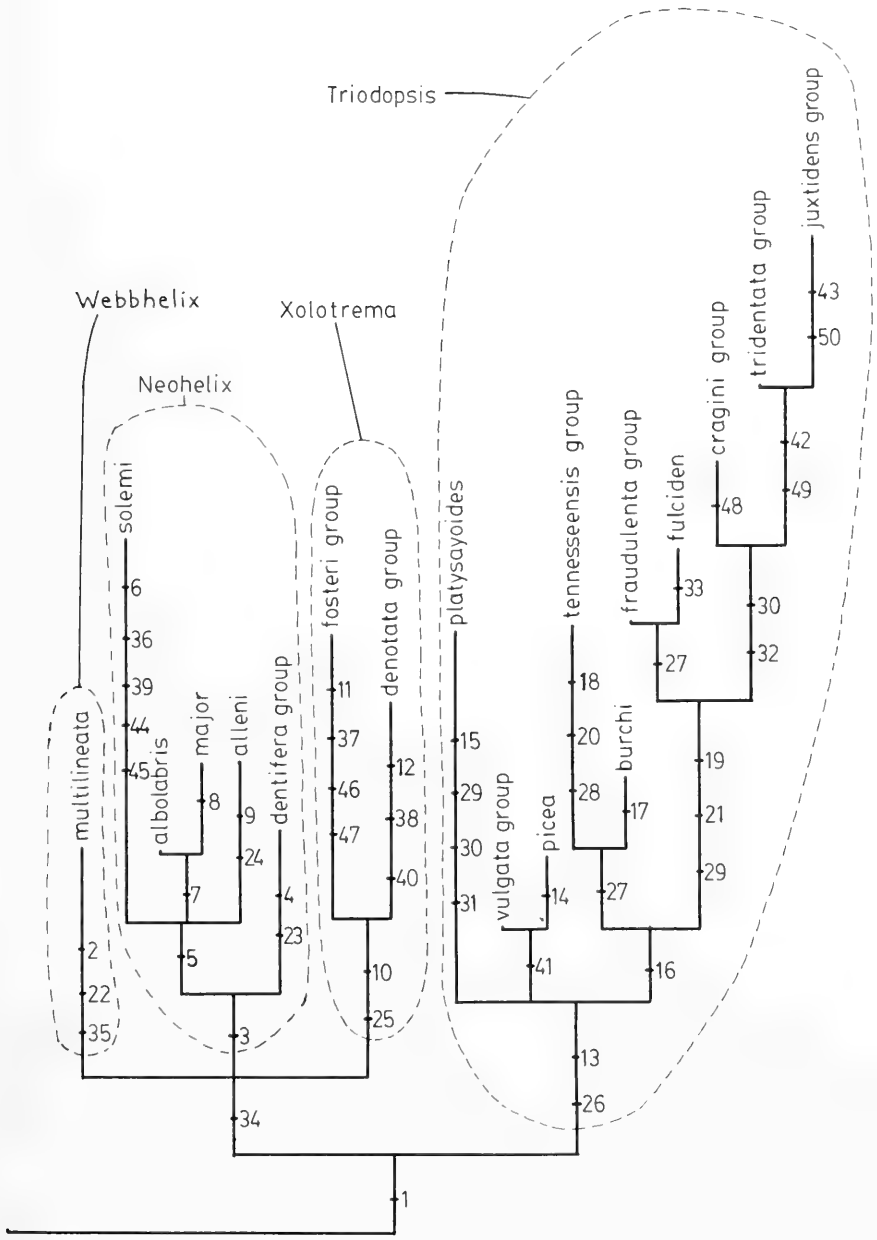

FIG. 24. "Anatomy Tree": a phylogenetic hypothesis for the eastern American triodopsines based on penial morphology (50 character-state transformations shown in Figs. 19–23). This is the single most parsimonious tree generated by PAUP, with a consistency index of .970.

having convergences in Transformations 29 and 30, so Fig. 24 was decidedly the best cladogram to fit the data.

Thus Fig. 24 is the "Anatomy Tree". The branch lengths of this cladogram are scaled to the number of transformations they contain, so are a rough indicator of the degree of evolutionary change in penial morphology.

ALLOZYMIC ANALYSES

Complete electrophoretic results are presented in Table 2. In this table, each allele (electromorph) is represented by its migration distance on the gel in mm relative to the control (*Mesodon zaletus* from Monte Sano, Alabama: FMNH 214772 and 214773), the migration distance of which was arbitrarily set at 100 mm. Seventy-four alleles were detected in the eastern triodopsines and 9 in the outgroup *Allogona profunda.* The most variable loci were Lap and Pgm, with 12 and 11 alleles. Sordh and Me each had 8 alleles; Icd had 7; Gpi had 5; Mdh-1, Mdh-2, Gd-1, Gd-2, and Sod-1 each had 4; Sod-2, Got-2, and Mpi each had three; Got-1 had two; and Pgd was the only monomorphic locus.

Heterozygosity within populations was extremely low. Most populations were monomorphic for all but two or three loci, with a maximum of three alleles per locus (Table 2).

Twenty triodopsine alleles were absent from the outgroup *Mesodon* and therefore were presumed apomorphous. The distributions of these alleles among triodopsine species are listed in Table 3. Twelve alleles were restricted to a single species; the remaining 8 were present in two to 10 species.

Phylogenetic analysis produced the "Alleles Tree" presented in Fig. 25. This cladogram is the consensus of the first 50 trees with a maximum, identical consistency index generated by PAUP; its numbered transformations refer to the alleles listed in Table 3. The Alleles Tree contains one convergence (Lap_{94} between *picea* and *tennesseensis*) and one reversal (loss of Icd_{98} in *hopetonensis*). Comparison of 50 trees showed that both this homoplasy and this reversal are robust, occurring in 100% and 88% of the trees respectively.

Phenetic analyses of the two independent subsets of the allozymic data are presented in Figs. 26 and 27. The first of these, the "Wagner-1 Tree", comprising 32 species evaluated over 16 loci, has a cophenetic correlations of .897, indicating only mild distortion of the original genetic distance matrix. The "Wagner-2 Tree" (21 species, 8 loci) has a similarly high cophenetic correlation of .883.

CONSENSUS PHYLOGENY

To aid in comparing the Anatomy Tree (Fig. 24), the Alleles Tree (Fig. 25), the Wagner-1 Tree (Fig. 26), and the Wagner-2 Tree (Fig. 27), each was labeled in a consistent manner: genera and outgroups were enclosed by dashed lines.

The trees were weighted for comparison. In the Anatomy Tree, 3 out of the 50 transformations (.06) showed reversal or convergence, whereas the Alleles Tree had 2 out of 20 (.10). Dividing these gave a "reliability" of anatomical over allozymic data units of 1.6. The number of data units for each tree was: Anatomy 50, Alleles 20, Wagner-1 73, and Wagner-2 28. Multiplying the morphological data units by 1.6 and dividing all by 75 and rounding gave the following weights: 1.0 for the Anatomy Tree, 0.3 for the Alleles Tree, 1.0 for the Wagner-1 Tree, and 0.4 for the Wagner-2 Tree.

The four genera—*Neohelix, Triodopsis, Webbhelix,* and *Xolotrema*—are distinct and coherent throughout all four Trees (Figs. 24–27). The four minor exceptions to this general pattern are readily resolved. (1) In the Alleles Tree, *N. albolabris* appears in *Triodopsis* due only to the presence of Icd_{98} (Transformation 10) in one of its three populations (*albolabris*-2), which could easily be a homoplasy. (2) Also in the Alleles Tree, *T. messana* is grouped in *Neohelix* due only to its possession of Icd_{96} (Transformation 11), which could be a homoplasy. (3) In the Wagner-1 Tree, *T. burchi* groups within *Neohelix;* in the equally weighted Anatomy Tree, however it pairs with the *T. tennesseensis* group, well within *Triodopsis,* and the occurrence of this pairing in the Alleles Tree gives it greater weight than a *Neohelix* position for *T. burchi;* its genetic similarity to *Neohelix* could be due either to homoplasy in alleles or to retention of plesiomorphous alleles. (4) The isolated position of *X. fosteri* within *Neohelix* in the Wagner-2 Tree (weight 0.4) is outweighed by its firm position within *Xolotrema* in both the Anatomy and Alleles Trees (combined weight 1.3); *X. fosteri* does not occur in the Wagner-1 Tree. With these

exceptions resolved, there remains no doubt of the robustness of the four genera.

Neohelix, Webbhelix, and *Xolotrema* together constitute a monophyletic group, according to the Anatomy Tree and both Wagner Trees. According to the Wagner-1 Tree, *Webbhelix* is the most plesiomorphous genus; in both the Anatomy and Alleles Trees, it is concordant with *Neohelix* and *Xolotrema,* but retains more plesiomorphous charcter-states than either of these genera. This combined evidence that *Webbhelix* is the most plesiomorphous genus of eastern triodopsines outweighs the Wagner-2 Tree's placement of it as sister group to *Xolotrema.*

The grouping of *Neohelix albolabris, N. major, N. alleni,* and the highly derived *N. solemi* in the Anatomy Tree receives enough verification in the two Wagner Trees for acceptance as it stands. In the Wagner-1 Tree, *alleni, major,* and *solemi* cluster together but are isolated from *albolabris.* However, in the Wagner-2 Tree, *albolabris* (two populations) does cluster with *alleni* (three populations) and *major* (two populations), but this cluster is isolated from a single population of *alleni* (*alleni*-4); *solemi* is missing from this tree. Thus, except for one slightly errant population in each of the two Wagner Trees, the evidence is consistent for an *albolabris-major-alleni-solemi* cluster, with *solemi* the most highly derived member both anatomically (Fig. 24) and electrophoretically (Fig. 26) as indicated by its long branch length in each of these trees. These four species comprise the *Neohelix albolabris* group, which is revised in the following section.

The *Neohelix dentifera* group *(dentifera, divesta,* and *lioderma)* is clearly coherent and isolated from the other *Neohelix* anatomically (Fig. 24) and electrophoretically (Figs. 26, 27). There is no evidence in any of these trees as to the relationships of the three species within the group, but *dentifera* appears to have a less apomorphous form of enlarged basal pustules, as discussed under anatomical Transformation 4, so may be plesiomorphous within the group. The *dentifera* group's position primitive to the *albolabris* group is evident in the Anatomy, Wagner-1, and Wagner-2 Trees, and is only contradicted by *alleni* and *lioderma* sharing Icd_{96} (Transformation 11) in the Alleles Tree, which could easily be a convergence and is strongly outweighed by the evidence of the other trees.

The anatomical division of *Xolotrema* (Fig. 24) into the *fosteri* group *(fosteri* and *occidentalis)* and the *denotata* group *(denotata, obstricta,* and *caroliniensis)* is only partially supported by the electrophoretic data. The pair *denotata* and *obstricta* is linked by a unique derived allele (Me_{108}) in the Alleles Tree (Fig. 25) and is also tightly linked in both Wagner Trees (Figs. 26, 27), but *caroliniensis* groups no closer to this pair than does *fosteri* in the Alleles Tree or *occidentalis* in both Wagner Trees. However, since *caroliniensis* is represented electrophoretically by only a single specimen (Table 2), its relative position in these trees should not be considered very precise. Complete electrophoretic data were lacking for *fosteri* (Table 2), so it does not occur in the Wagner-1 Tree. In the Wagner-2 Tree, *fosteri* is strongly isolated not only from *occidentalis* but from the remainder of *Xolotrema* in general, but this placement based on genetic distance is shown cladistically to be aberrant in the Alleles Tree: *fosteri* shares one derived allele, ($Sordh_{98}$) with the remainder of *Xolotrema* and another derived allele (Me_{105}) with the *denotata* group. The Alleles Tree also shows that *occidentalis* is separated from *fosteri* by its lack of Me_{105} and its unique possession of Gpi_{107}.

Despite these partial discrepancies with the electrophoretic trees, the anatomical disjunction between the *fosteri* and *denotata* groups is so extreme (seven transformations in the anatomy Tree) and the penial sculpture within each of these two groups is so cohesive (Figs. 7 and 8), that there can be no doubt of their separation. The fact that there has been little electrophoretic differentiation between the *fosteri* and *denotata* groups suggests that their anatomical distinctions have evolved relatively recently. There is no clear evidence from any of the trees as to which of these two groups is the more plesiomorphous.

Within *Triodopsis* there are some discrepancies among the four trees of such magnitude that the original dissections were reexamined and several interpretive errors were detected. The Consensus Tree (Fig. 28), therefore, differs from the Anatomy Tree (Fig. 24) more for this genus than for any other, and contains some redefinitions of species groups.

In the Wagner-1 Tree (Fig. 26), the *T. vulgata* group *(vulgata* and *claibornensis), T. picea,* the *T. fraudulenta* group (*fraudulenta* and *rugosa,* but represented only by *fraudulenta*), and *T. platysayoides* form a single, shallowly rooted group with no differentiation into subgroups except for a shallow pairing of

TABLE 2. Allozyme data for 64 populations comprising 35 species of eastern American triodopsines, plus two populations of the outgroup *Allogona profunda*. Not shown in the table is a 16th locus, Pgd, for which all populations were monomorphic at 100.

Species	FMNH catalogue number	N	Locus														Total alleles
			Sordh	Mdh-1	Mdh-2	Me	Icd	Gd-1	Gd-2	Sod-1	Sod-2	Got-1	Got-2	Pgm	Lap	Mpi	Gpi	
Allogona profunda	—	10	94	107	100(.20) 95(.80)	104	90	103(.10) 99(.90)	99	110(.05)	100	100	97	98(.15) 95(.75) 86(.10)	105(.85) 102(.10) 98(.05)	100	104(.70) 100(.30)	
T. alabamensis	214791	6	102	96	100	100	100	102	98	90	100	100(.58) (.42)	97	103(.08) 99(.84) 97(.08)	99 99	102 102	104(.08) 100(.75) 95(.17)	
N. albolabris	214920	5	103	91	100	100	100	103	99	90	107	100	97	95(.80) 91(.20)	99(.20) 97(.80)	100	100	
N. alleni	214911	6	103(.58) 101(.17) 89(.25)	91	100	100	96	103	99	100	107	100	97	99(.58) 97(.42)	99	100	100	
T. anteridon	214793	3	102	96	100	99	98	102	99	100(.67) 90(.33)	100	100	97	99(.33) 97(.67)	97	102	104(.17) 100(.83)	
T. burchi	214797	6	102	96	100	102	100	102	99	100	107	100	97	102(.75) 100(.25)	97	100	100	
X. caroliniensis	—	1	98	91	99	105(.50) 100(.50)	103	103	99	90	107	100	97	97	102	100	100	
T. claibornensis	214800	4	101	96	100	100	98	102	99	100(.88) 90(.12)	100	100	97	97(.50) 95(.50)	99	102	100	
T. complanata	214802	2	101	96	100	102	100	102	99	90	100	100	97	99(.75) 97(.25)	97	102	104(.50) 100(.50)	
T. cragini	214803	6	102	96	100	100	102	102	99	90	100	97	97	99	97	100	100(.08) 95(.92)	
X. denotata	214806	9	98	91	99	108(.06) 105(.94)	103	103	100	90	107	100	97	97(.94) 95(.06)	102(.06) 100(.22) 97(.72)	100	104(.06) 100(.94)	
N. dentifera	214809	5	102	96	100	100	102	103	99	100	107	100	97	97(.10) 95(.50) 91(.40)	97(.40) 95(.60)	96	100	
N. divesta	214814	5	102	96	100	100(.70)	100	103	99	100	107	100	97	97	99	96	100	
T. fraudulenta	214822	6	102	96	99.5	104(.83) 100(.17)	98	102	100	100(.50) 90(.50)	107(.33) 10(.67)	97	97	97(.08) 95(.92)	99(.08) 97(.75) 95(.17)	100(.92) 96(.08)	100	
T. hopetonensis	—	4	102	96	100	98	100	102	100	90	100	100	97	75	98	102	104(.75) 100(.25)	
T. juxtidens	214842	5	100	96	100	98	98	102	100	100(.10) 90(.90)	100	100	97	97	99(.80) 97(.20)	102	104(.50) 100(.50)	
N. lioderma	214844	10	100	96	100	97	96	103	99	100	107	100	97	97(.15) 95(.85)	99	96	100	
N. major	214930	5	103	91	100	100	100	103	99	100	107	100	97	98(.50) 95(.50)	99(.90) 97(.10)	100	100	
T. messana	214846	4	102	96	100	100	96	102	99	90	100	100(.88) (.12)	97	97(.75) 95(.25)	97	102	104(.25) 100(.75)	
W. multilineata	214849	3	102	91	99	100	100	101	100	100	100	100	97	98	100	96	99	
T. neglecta	214850	5	102	96	100	98	100	102	98	100(.10)	100	100	105(.30) 97(.70)	100(.20) 98(.70) 91(.10)	99(.10) 97(.90)	102(.20) 100(.80)	100	

X. obstricta	214854	5	98	91	99	108(.10) 105(.90)	103	103	100	90	107	100	97	99(.10) 97(.70) 95(.20)	97	100	104(.10) 100(.90)
X. occidentalis	214856	5	98	91	99	100	103	103	100	90	107	100	97	97	95	100	107(.80) 100(.20)
T. palustris	214857	5	102	96	100	100	100	102	99	90	100	100	97	100(.20) 98(.40) 95(.40)	99(.70) 97(.30)	102(.50) 100(.50)	100
T. pendula	214859	4	102(.88) 100(.12)	96(.25) 91(.75)	100	98	98	102	100	90	100	100	97	75	100(.88) 99(.12)	102	100
T. picea	214860	5	102	91	100	104(.70) 100(.10) 98(.20)	98	102	100	100	107(.30) 100(.70)	100(.10) 97(.90)	97	100(.10) 97(.70) 95(.20)	97(.10) 94(.40) 92(.50)	102	100
T. platysayoides	214861	4	101	96	100	100	102	102	98	100	100	97	97	97(.62) 95(.38)	97	102	100
N. solemi	214943	3	101.5	91	100	100	100	103	99	100	106	100	97	98(.50) 95(.50)	97	100	95
T. tennesseensis	214864	3	101	96	100	102	100	102	99	100(.17) 90(.83)	100	100(.83) 97(.17)	97	100(.17) 97(.66) 95(.17)	97(.33) 94(.67)	102	100
T. tridentata	214876	4	101	96	100	99	100	102	100	90	100	100	97	98(.12) 96(.88)	99	102	100
T. vannostrandi	214880	7	102	96	100	98	98	102	99	100(.07) 90(.93)	107(.07) 100(.93)	100	97	97(.21) 95(.79)	100(.86) 98(.14)	102	100(.71) 95(.29)
T. vulgata	214884	7	101	96	100	104(.07) 100(.86) 98(.07)	98	102	100	100(.64) 90(.36)	100	100	100(.36) 97(.64)	98(.43) 95(.57)	98(.93) 89(.07)	102	100(.79) 95(.21)
T. vultuosa	214887	3	102	96	100	98	102	102	99	90	100	97	97	103(.66) 100(.17) 99(.17)	98	102	100(.50) 95(.50)
Allogona profunda—2	—	2	94	107	100(.50) 95(.50)	104	90	—	—	99	—	100	—	91(.75) 86(.25)	—	100	104(.25) 100(.75)
N. albolabris—2	—	2	103	96(.25) 91(.75)	100	100	98	—	99	90	107	100	97	95(.75) 91(.25)	97	—	100
N. albolabris—3	214919	4	103	96	100	100	100	103	99	100(.37) 90(.63)	107	100	97	95	99	102	100
N. alleni—2	214908	1	100	96	100	100	—	—	—	100	107	100	97	95	—	100	100
N. alleni—3	214909	1	100	96	100	100	—	—	—	100	107	100	97	95	—	100	100
N. alleni—4	—	12	102	96	100	100	100	103	100	100	107	100	100	97	99	100	100
N. alleni—5	214910	5	103(.70) 101(.20) 89(.10)	96(.10) 91(.90)	100	100	96	103	98	100(.75) 90(.25)	107	100	97	97(.60) 95(.30) 91(.10)	99(.60) 96(.40)	100	104(.20) 100(.80)
T. anteridon—2	214796	2	102	96	100	99	98	102	99	100(.75) 90(.25)	100	100	97	99(.25) 97(.75)	97	100	100
T. cragini—2	214804	3	102	96	100	100	100	102	99	90	100	100	97	97(.50) 95(.50)	97	102	100
X. denotata—2	214805	2	98	91	99	105	—	—	—	90	107	100	—	97	—	100	104(.25) 100(.75)
N. dentifera—2	214810	1	102	96	100	100	102	103	99	90	107	100	97	95	97(.50) 95(.50)	96	100
N. divesta—2	214813	2	102	96	100	100	100	103	99	100	107	100	97	95	99(.75) 96(.25)	96	100

TABLE 2. (Continued)

Species	FMNH catalogue number	N	Locus														Total alleles
			Sordh	Mdh-1	Mdh-2	Me	Icd	Gd-1	Gd-2	Sod-1	Sod-2	Got-1	Got-2	Pgm	Lap	Mpi	Gpi	
*X. fosteri	214819	10	98	96	100	105(.45) 100(.55)	100	—	—	100	—	100	97	97(.25) 95(.25) 91(.50)	97	100	100	
*T. fulciden	214823	2	102	96	100	104	98	—	—	90	100	100	97	103(.50) 100(.50)	101(.75) 98(.25)	102	95	
*T. henriettae	214824	2	102	96	100	98	104(.75) 102(.25)	—	—	90	100	97	97	100(.50) 98(.50)	98	102	95	
T. hopetonensis—2	214832	1	102	96	100	98	100	102	100	90	100	100	97	75	98	102	100	
T. juxtidens—2	214840	2	101	96	100	98	98	102	100	90	100	100	97	100(.50) 97(.50)	99	102	100	
N. major—2	214928	3	103	91	100	100	100	103	97	100	107	100	97	98(.33) 95(.67)	97	102(.17) 100(.83)	100	
N. major—3	214927	7	103	96(.79) 91(.21)	100	100(.30) 98(.70)	100	103	99	100	107	100	100	95	97	100	100	
W. multilineata—2	214848	1	102	91	99	100	100	—	100	100	107	100	97	98	100	96	100	
X. obstricta—2	214852	1	98	91	99	100	—	—	—	90	—	100	—	97(.50) 95(.50)	—	100	100	
X. obstricta—3	214853	1	98	96	99	100	—	—	—	90	—	100	—	97(.50) 95(.50)	—	100	104	
X. occidentalis—2	214855	2	98	91	99	100	103	103	100	100(.50) 90(.50)	107	100	97	97	95	100	100	
T. palustris—2	214858	2	102	96	100	100	100	102	99	90	100	100	97	100(.25) 98(.50) 95(.25)	99(.50) 97(.50)	102	100	
T. tennesseensis—2	214865	2	101	96	100	102	100	102	99	90	100	100	97	100(.75) 95(.25)	94	102	100	
T. tridentata—2	—	2	101	96	100	99	—	—	—	90	—	100	—	99(.25) 97(.50) 95(.25)	—	—	100	
T. tridentata—3	214866	1	101	96	100	100	102	—	99	100(.50) 90(.50)	100	100	97	98(.50) 96(.50)	96	97	100	
T. tridentata—4	214878	3	101	100(.17) 96(.83)	100	99	103(.33) 100(.67)	102	99	100(.17) 90(.83)	100	100	97	99(.50) 97(.50)	99(.83) 97(.17)	102	100	
T. tridentata—5	214867	1	101	96	100	—	100	—	—	90	100	100	97	97	97	100	100	
T. tridentata—6	—	9	101	96	100	—	100	—	—	90	100	100	97	99(.44) 97(.56)	97	100	100	
T. vulgata—2	214885	3	101	96	100	100	98	102	100	100(.67) 90(.33)	100	100(.83) 97(.17)	100(.67) 97(.33)	98(.33) 95(.67)	98	102	104(.17) 100(.83)	
T. vultuosa—2	—	2	102	96	100	98	102	102	99	90	100	97	97	97(.50) 95(.50)	98	102	100(.50) 95(.50)	
T. vultuosa—3	—	2	102	96	100	98	102	102	99	90	100	97	97	103(.75) 100(.25)	98	102	100(.50) 95(.50)	
Total Alleles: Triodopsines			7	3	3	8	6	3	4	2	3	2	3	10	11	3	5	73
Total Alleles: *Allogona* only			1	1	1	0	1	1	0	2	0	0	0	1	1	0	0	9
Total			8	4	4	8	7	4	4	4	3	2	3	11	12	3	5	82

TABLE 3. Alleles in eastern American triodopsines which are considered apomorphous (i.e., absent from their outgroup *Mesodon*), and the species in which they were detected.

Locus	Allele	Species
1. Sordh	101.5	*solemi*
2. Sordh	98	*caroliniensis, denotata, fosteri, obstricta, occidentalis*
3. Sordh	89	*alleni*
4. Mdh-2	99.5	*fraudulenta*
5. Me	108	*denotata, obstricta*
6. Me	105	*caroliniensis, denotata, fosteri, obstricta*
7. Me	102	*burchi, complanata, tennesseensis*
8. Me	97	*lioderma*
9. Icd	104	*henriettae*
10. Icd	98	*albolabris, anteridon, claibornensis, fulciden, juxtidens, pendula, picea, vannostrandi, vulgata*
11. Icd	96	*alleni, lioderma, messana*
12. Gd-1	101	*multilineata*
13. Gd-2	97	*major*
14. Sod-2	106	*solemi*
15. Got-2	105	*neglecta*
16. Pgm	96	*tridentata*
17. Pgm	75	*hopetonensis, pendula*
18. Lap	94	*picea, tennesseensis*
19. Gpi	107	*occidentalis*
20. Gpi	99	*multilineata*

fraudulenta and *picea.* This pattern contrasts strongly with that seen in the Anatomy Tree (Fig. 24), in which the *fraudulenta* group plus *fulciden* is quite isolated from the *vulgata* group, *picea,* and *platysayoides,* with the *tennesseensis* group plus *burchi* intervening. Selected dissections of *vulgata, picea, fraudulenta, rugosa, fulciden,* and *platysayoides* were repinned and compared. It was found that Fig. 10 misrepresents the pilastral structure of *fraudulenta,* which is actually much more like that of *picea* (Fig. 9b), but is variable within a single population (FMNH 214822), with some individuals (e.g. #6) showing secondary fusion of pustules which only superficially resembles the knob-less, spurred pilastral polygons of the *tridentata, juxtidens,* and *cragini* groups. Also, the pore of *fraudulenta* is Type-2 ventrally subterminal, which, in retrospect, is apparent in Fig. 10. Thus, *fraudulenta* is actually anatomically closest to *picea,* and the basal merging of its ventral-most wall columns is homoplasic with this condition in *rugosa* and *fulciden,* which have the *tridentata*-like, rather than the *picea*-like pilastral sculpture, and which still appear to have a terminal pore. This dissolves the previous *fraudulenta* group *(fraudulenta* and *rugosa)* of Table 1 and Figs. 24–27, leaving *rugosa* by itself, and redefines a new two-species *fradulenta* group as *picea* and *fradulenta,* with *fraudulenta* its most derived member, and closest anatomically to the *vulgata* group. On re-inspection, it appears that *platysayoides's* unique pilastral sculpture could be directly evolved from the more undifferentiated sculpture of the pilaster *vulgata* and *claibornensis,* as was anticipated in the discussion of Transformation 15. These revised anatomical decisions are reflected in the positions of the (revised) *vulgata* group and *platysayoides* in Fig. 28, which, unlike the Anatomy Tree (Fig. 24), is compatible with the Wagner-1 Tree (Fig. 26). Only one of these reconsidered species *(vulgata)* occurs in the Wagner-2 Tree (Fig. 27), and its pairing there with *tridentata* is at variance with the consensus reached in Fig. 28, but carries little relative weight. The Alleles Tree (Fig. 25) offers no strong contradiction to the Consensus Tree's arrangement of the *vulgata* group (it lacks *platysayoides*), for *fraudulenta* only lacks one allele (Icd_{98}) which is shared by *vulgata, picea,* and *claibornensis,* and the value of this allele is apparently lessened by its patchy distribution among members of the *tridentata* and *juxtidens* groups as well.

The *T. tennesseensis* group *(tennesseensis* and *complanata)* is consistently supported by the three trees (Anatomy, Alleles, and Wagner-1) which contain both species. Its grouping with *T. burchi,* evident in the Anatomy and Alleles Trees (combined weight = 1.3), is strongly contradicted by the Wagner-1 Tree (weight = 1.0), in which *burchi* appears between *Webbhelix* and the *Neohelix-Xolotrema* lineage. Dissections of *T. burchi* were reexamined, but no evidence was found for reinterpreting it anatomically; therefore in the Consensus Tree (Fig. 28) *burchi* is retained next to the *tennesseensis* group with a question mark denoting its problematic status. The position of the *tennesseensis* group-?*burchi* lineage as sister group to the *vulgata* group *platysayoides* lineage is clear-cut on both the Anatomy and Wagner-1 Trees (combined weight = 2.0) and is only mildly contradicted by the grouping of *tennesseensis* with *tridentata* and *juxtidens* in the Wagner-2 Tree (weight = 0.4).

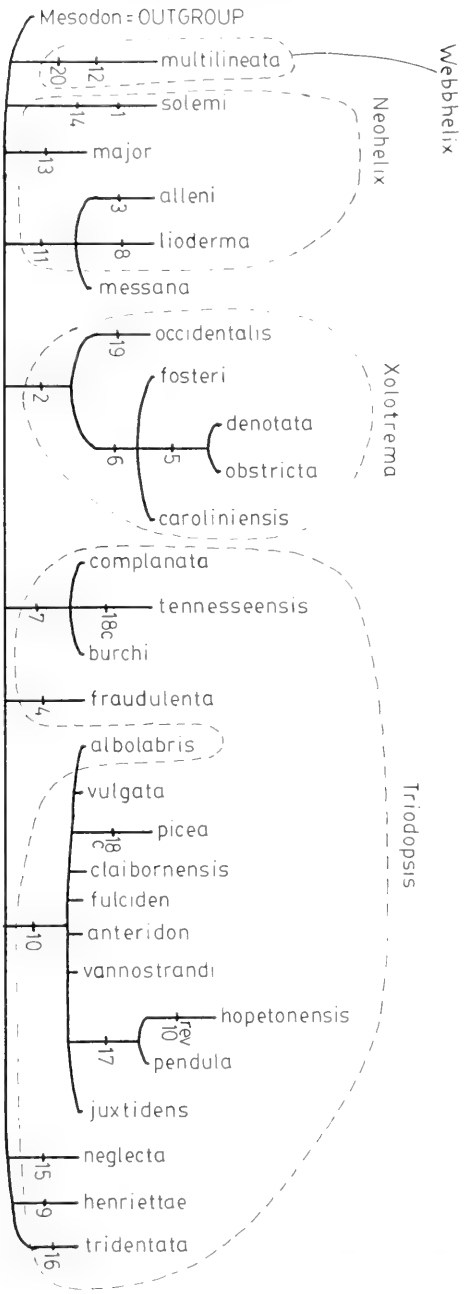

FIG. 25. "Alleles Tree": a phylogenetic hypothesis for the eastern American triodopsines based on allozymes, with *Mesodon* as outgroup. The 20 uniquely derived alleles are listed in Table 3. This tree is the consensus of 50 trees of equal and maximum parsimony generated by PAUP, with a consistency index of .950.

The phylogeny of the remainder of *Triodopsis* is fairly consistent among the four trees, and generally supports the Anatomy Tree (Fig. 24). The *tridentata* group *(tridentata, anteridon, fallax, obsoleta, hopetonensis, palustris, messana, alabamensis,*

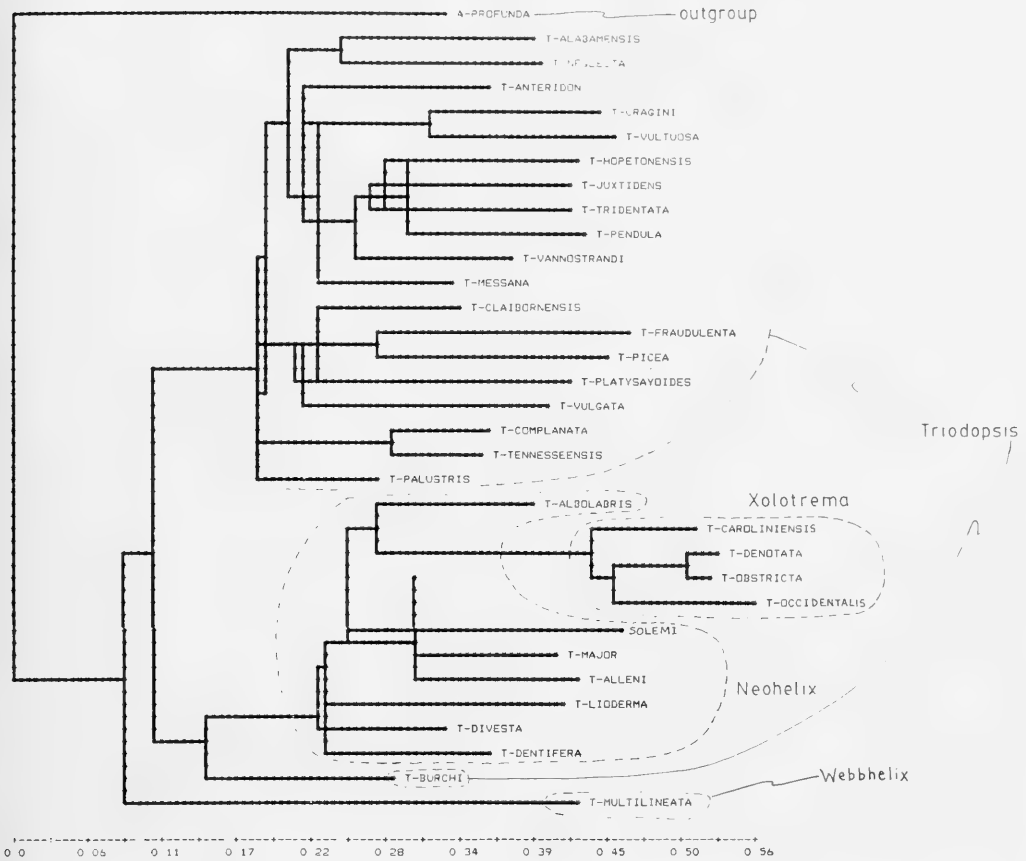

FIG. 26. "Wagner-1 Tree": a distance-Wagner tree for 32 species of eastern American triodopsines, with *Allogona* as outgroup. Computed from the Prevosti distance matrix based on 16 allozymic loci (Table 2, upper half). The cophenetic correlation is .897. The branch lengths are optimized.

vannostrandi, and *soelneri)* and the *juxtidens* group *(juxtidens, discoidea, pendula,* and *neglecta)* cannot be distinguished electrophoretically (Figs. 25–27); nevertheless their separation is accepted on both anatomical and biogeographic grounds. The differences in pore position and peduncle size between the *tridentata* and *juxtidens* groups, although not extreme and not always easy to detect in dissection, are disjunct (see previous discussions under Transformations 42, 43, 49, and 50). Biogeographically, none of the four species of the *juxtidens* group (0%) show range overlap (Fig. 49), whereas there are approximately 10 range overlaps among the 10 species of the *tridentata* group (10/ (10 take 2) = 10/45 = 22%); this is consistent with the view that the *juxtidens* group is more recently evolved and less differentiated. The lack of electrophoretic differentiation between the *tridentata* and *juxtidens* groups is interpreted as evidence that their split was relatively recent.

The *T. cragini* group *(cragini, vultuosa,* and *henriettae)* is tightly coherent in both the Anatomy and Wagner-1 Trees (Figs. 24, 26), although *henriettae* is missing from the latter. In the Wagner-2 Tree (Fig. 27), *henriettae* and two populations of *vultuosa* cluster closely, with *cragini* more distantly connected and with *fulciden* intervening, which is discussed below. The consensus of these three trees (the Alleles Tree contains no information beyond *henriettae's* possession of the unique, derived allele lcd_{104}) is that the *cragini* group is well defined and that *cragini* is

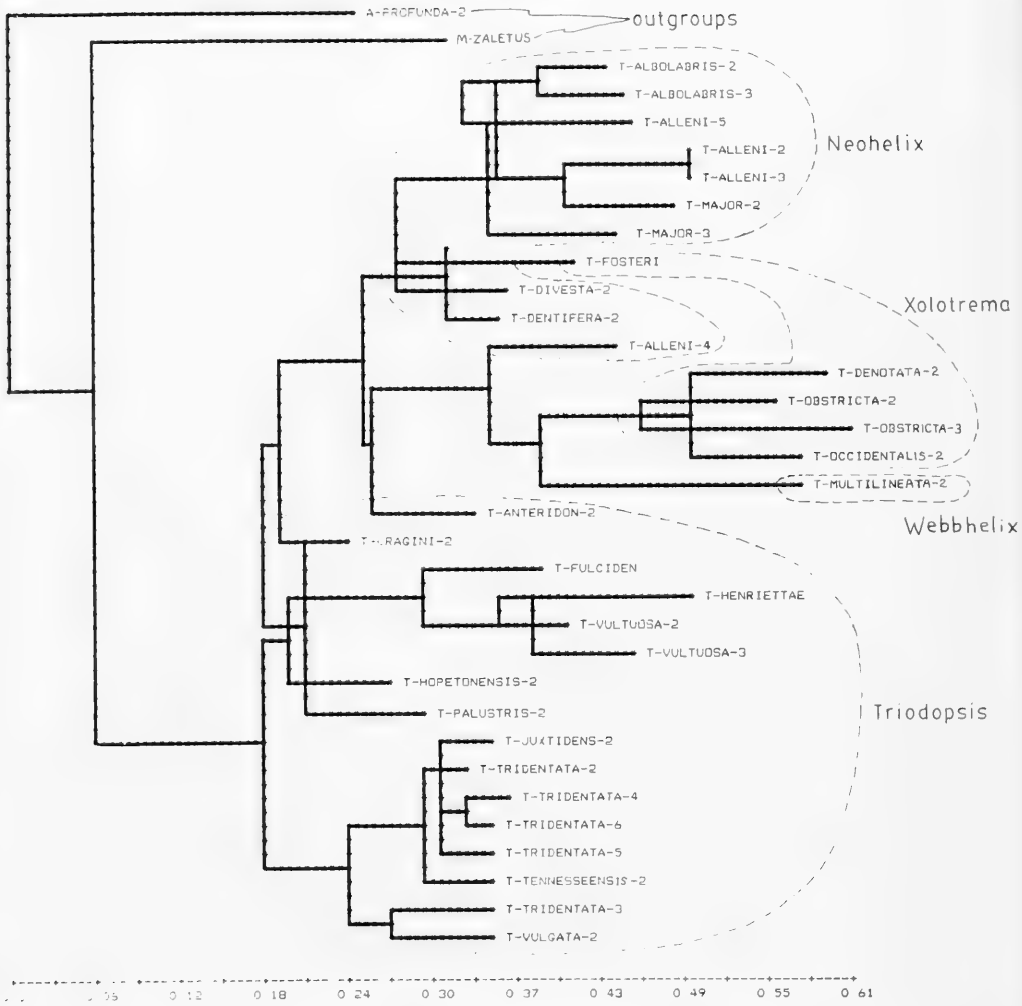

FIG. 27. "Wagner-2 Tree": a distance-Wagner tree for 3 additional species and 29 additional populations of 18 of the species of eastern American triodopsines represented in the Wagner-1 Tree (Fig. 26), with *Allogona profunda* and *Mesodon zaletus* as outgroups. Computed from the Prevosti distance matrix based on the 8 allozymic loci for which all populations had complete data (Table 2, lower half). The cophenetic correlation is .883; the branch lengths are optimized.

probably the most plesiomorphous species of the group. The position of the *cragini* group is outside the *tridentata-juxtidens* lineage in the Anatomy Tree (weight 1.0), shallowly within this lineage in the Wagner-1 Tree (weight 1.0), and outside this lineage in the Wagner-2 Tree (weight 0.4). The consensus, therefore, is the separation of these lineages as sister groups.

Complete electrophoretic data were lacking for *T. fulciden*, and none were available for *T. rugosa*, so the only test of their paired position in the Anatomy Tree (in which *rugosa* equals the "*fraudulenta* group"), is *fulciden's* position in the Wagner-2 Tree. Its close relationship to the *cragini* group in this tree in entirely supportive of its being a sister group to the *cragini-tridentata-juxtidens* lineage (Fig. 24), but could also denote its being a sister group of the *cragini* group alone. In the Consensus Tree (Fig. 28), therefore, the *fulciden-rugosa* pair (named the *rugosa* group) is positioned as in the Anatomy Tree (Fig. 24), but with a question mark. Since data for *rugosa* are

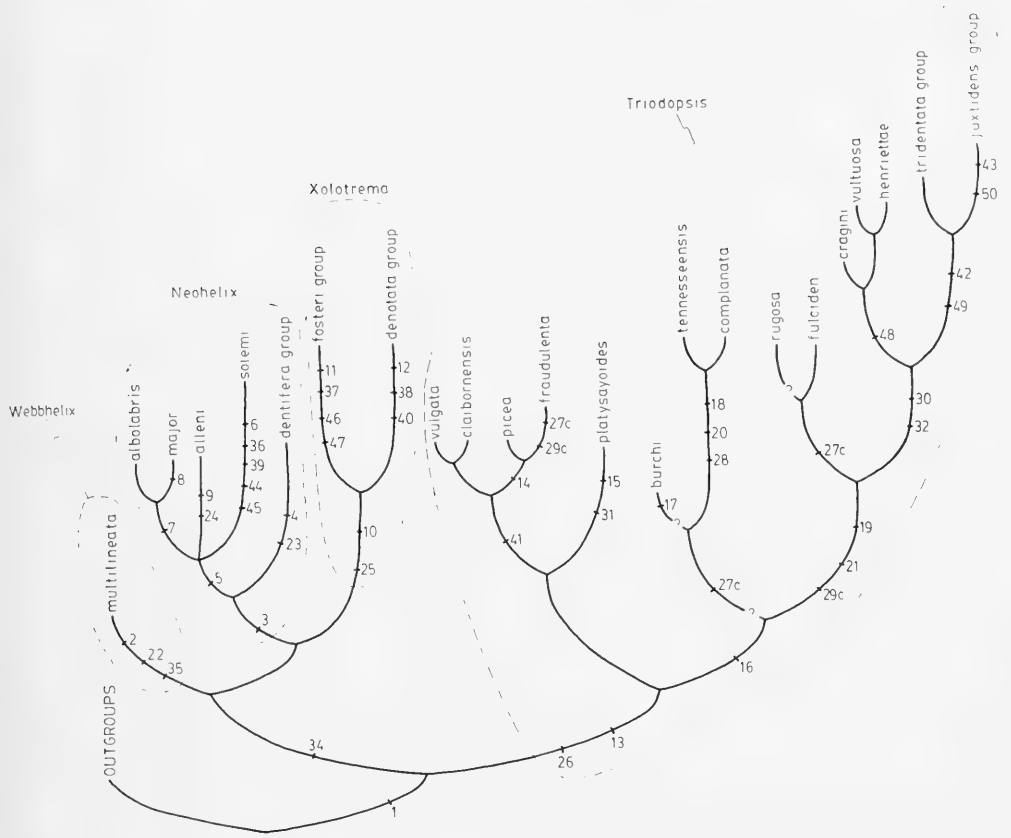

FIG. 28. "Consensus Tree": the robust phylogenetic hypothesis for the eastern triodopsines, representing the weighted consensus of the Anatomy, Alleles, Wagner-1, and Wagner-2 Trees (Figs. 24–27).

scant, this pairing is also uncertain, so *rugosa's* position in is also marked with a question mark.

The completed Consensus Tree for the eastern American triodopsines is presented in Fig. 28, labeled with the suggested anatomical charcter-state transformations as reassessed in the light of electrophoretic evidence. The Consensus Tree carries two convergences in transformation 27 and a single convergence in transformation 29. It represents a robust consensus between electrophoretic and anatomical data.

CONCHOLOGICAL VARIATION

Conchological illustrations of eastern American triodopsine species are presented in Figs. 29–45. Also included for comparative purposes is an illustration of *Allogona profunda,* the only eastern ashmunelline (Fig. 46a–b). Shell variation of eastern triodopsines has been thoroughly discussed by Pilsbry (1940), Vagvolgyi (1968), and Grimm (1975). An illustrated key to most of the species is contained in Burch (1962). It is important to remember when identifying any eastern American triodopsine that many species of the polygyrine genus *Mesodon* have closely convergent shells.

REVISION OF THE *NEOHELIX ALBOLABRIS* GROUP

The following classification is proposed, based on analyses of penis and shell. The complete systematic review is presented in Appendix B.

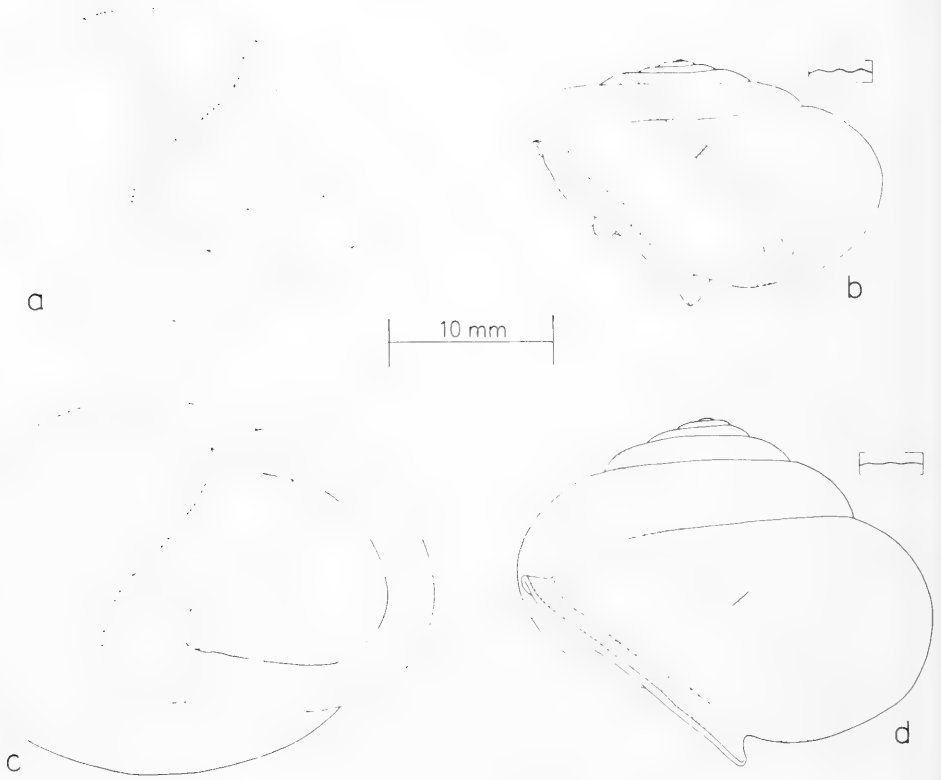

FIG. 29. Shells. **a–b.** *Neohelix dentifera* (Binney, 1837). FMNH 214810 #8. **c–d.** *Neohelix albolabris albolabris* (Say, 1816). FMNH 214920 #14.

albolabris group
albolabris
albolabris albolabris (Say, 1816)
albolabris bogani Emberton, new subspecies
major (Binney, 1837)
alleni group
alleni
alleni alleni (Sampson, 1883)
alleni fuscolabris (Pilsbry, 1903)
solemi Emberton, new species

Genitalic analysis

Species identification of each of the 46 populations (Fig. 47) was made by comparing its penial morphology with Figs. 2d, 3, 4, and 6b. Differences among *albolabris, alleni, major,* and *solemi* in upper penial sculpture were extremely stable over their geographical ranges, which made identifications easy and straightforward. For 39 of the populations, penial sculpture was examined by dissecting one to three specimens per population; for two populations (numbers 5, 32), specimens had partially everted their penes in the drowning jar, so could be identified without dissection; the remaining 5 populations (numbers 16–19, 27) were identified from published anatomical illustrations (Simpson, 1901; Pilsbry, 1940; Webb, 1952, 1954a).

The results of the penial-morphological measurements are presented in Table 4 as ranges over the three measured populations (one specimen per population). For each of the 7 variables, value ranges are underlined which do no overlap the value range of *albolabris albolabris.* Because of the small sample sizes and non-normal distributions, these differences were not tested for statistical significance. Between the two subspecies of *albolabris* there was no difference detected in any of the 7 penial-morphological variables, therefore they were pooled for cladistic analysis.

From Table 4, seven new penial-morphological transformations (transformations A–G) are proposed for a cladistic analysis of *albolabris, alleni, major,* and *solemi,* using

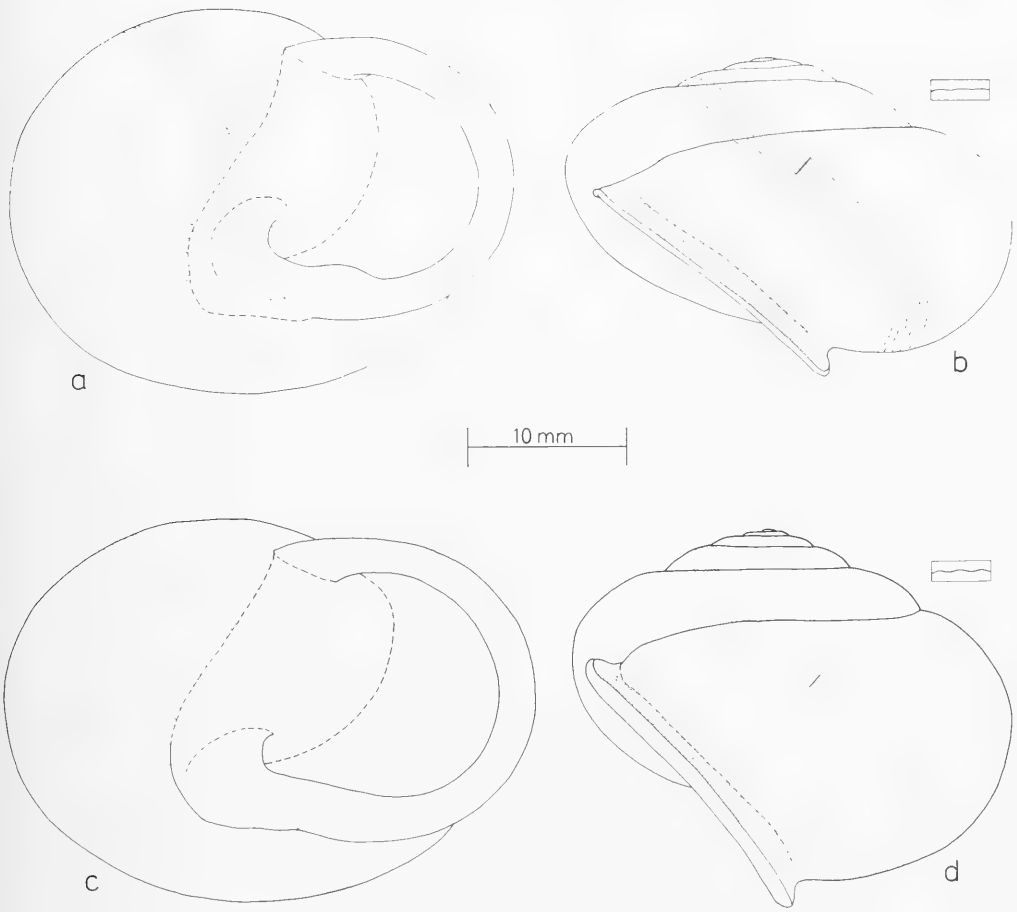

FIG. 30. Shells. **a–b.** *Neohelix alleni alleni* (Sampson, 1883). FMNH 214913 #B. **c–d.** *Neohelix major* (Binney, 1837). FMNH 214933 #H.

Webbhelix and the *dentifera* group as outgroups (Fig. 28). The format used is the same as used previously for transformations 1–50.

Transformation A—Preceding transformations: none.

Plesiomorphous state: penis, pilastral lappets, and wall pustules all moderate in size: penis length variable with median ca 13–14 mm, pilastral lappets less than .3 mm high, wall pustules less than .15 mm wide. Present in (outgroups): *Webbhelix multilineata* (penis and pustules), *N. dentifera* group (penis, lappets, upper pustules), *albolabris, alleni, solemi* (penis, lappets, basolateral pustules).

Apomorphous state: penis, pilastral lappets, and wall pustules all large: penis length invariable at 17 mm, pilastral lappets higher than .5 mm, wall pustules wider than .16 mm. Formerly and now present in: *major.*

Discussion. The penis of *major* (Fig. 4a) looks much like a hypertrophied version of *albolabris's* (Fig. 2d), so it appears that its longer penis and larger pilastral lappets and wall pustules are intercorrelated features of a general enlargement. Of the outgroups, all have a moderate penis length. Only *albolabris* (Fig. 2d), *alleni* (Fig. 3a, c), and *solemi* (Fig. 6b) have the plesiomorphous lappet height, since *Webbhelix multilineata* (Fig. 6a) lacks lappets and the *dentifera* group (Figs. 2a, 5a, d) has them doubled (Transformation 4). The plesiomorphous wall pustule size is unmodified only in *W. multilineata* (Fig. 6a), *albolabris* (Fig. 2d), and *alleni* (Fig. 3a, c); the

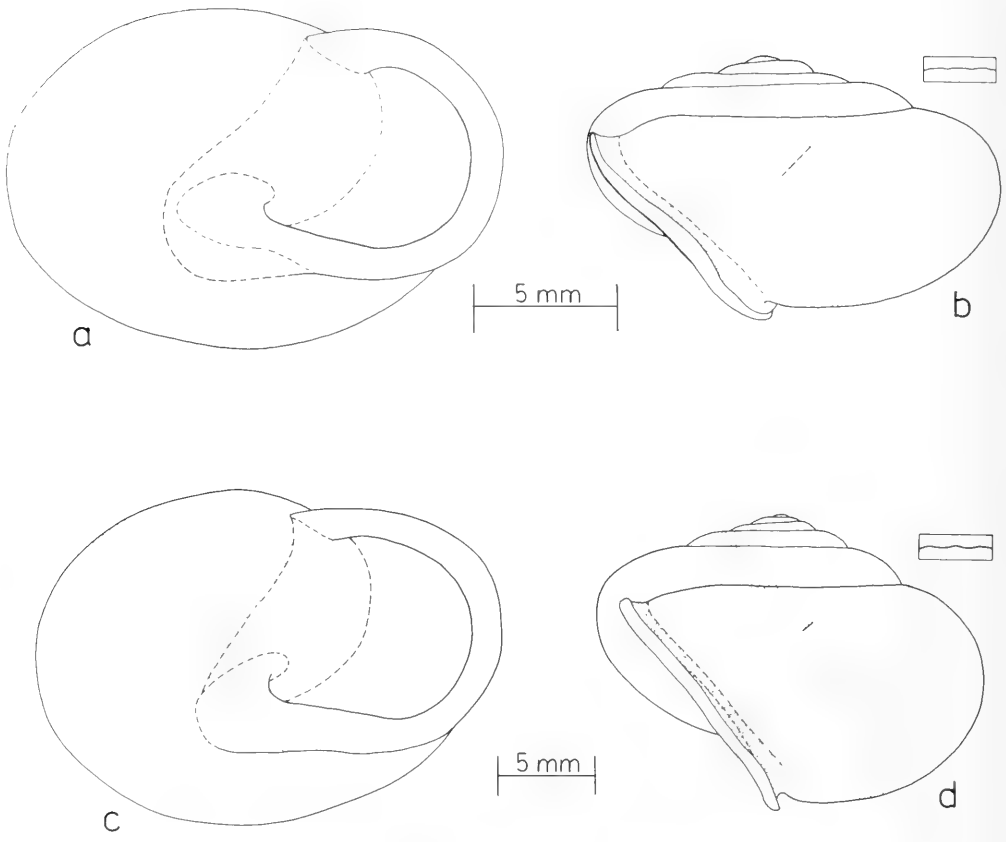

FIG. 31. Shells. **a–b.** *Neohelix lioderma* (Pilsbry, 1902). FMNH 214844 #A. **c–d.** *Neohelix divesta* (Gould, 1848). FMNH 214813 #A.

wall pustules are enlarged basally (Transformation 23) in the *dentifera* group (Figs. 2a, 5a, d) and enlarged everywhere but baso-laterally (Transformation B, below) in *solemi* (Fig. 6b).

Transformation B—Preceding transformations: none.

Plesiomorphous state: all wall pustules moderate and approximately equal in size, less than 0.15 mm wide. Present in (outgroups): *W. multilineata* (Fig. 6a), *albolabris* (Fig. 2d), *alleni* (Fig. 3a, c).

Apomorphous state: all but the baso-lateral wall pustules large, wider than .20 mm. Formerly and now present in *solemi* (Fig. 6b).

Discussion. Large wall pustules, 5–6 per 1.3 mm, occur in both *major* and *solemi* (Table 4, column 3), but this appears to be due to convergence. The large wall pustules of *solemi* (Fig. 6b) are neither uniformly sized nor accompanied by a large penis and large pilastral lappets, as they are in *major* (Fig. 4a).

Transformation C—Preceding transformations: none.

Plesiomorphous state: pilastral lappets about as wide as the wall pustules. Present in (outgroup): *alleni* (Fig. 3a, c), *solemi* (unmodified baso-lateral wall pustules: Fig. 6b).

Apomorphous state: pilastral lappets approximately twice as wide as the wall pustules. Formerly and now present in: *albolabris* (Fig. 2d), *major* (Fig. 4a).

Discussion. According to Table 4 (third and fourth columns), the pilastral lappets are slightly less dense than the columns of wall pustules in *alleni* (15–18 vs. 18–22 per 2.6 mm), and slightly more dense than the enlarged, central columns of wall pustules in *solemi* (14–15 vs. 8–12 per 2.6 mm). In contrast, the pilastral lappets in *albolabris* are

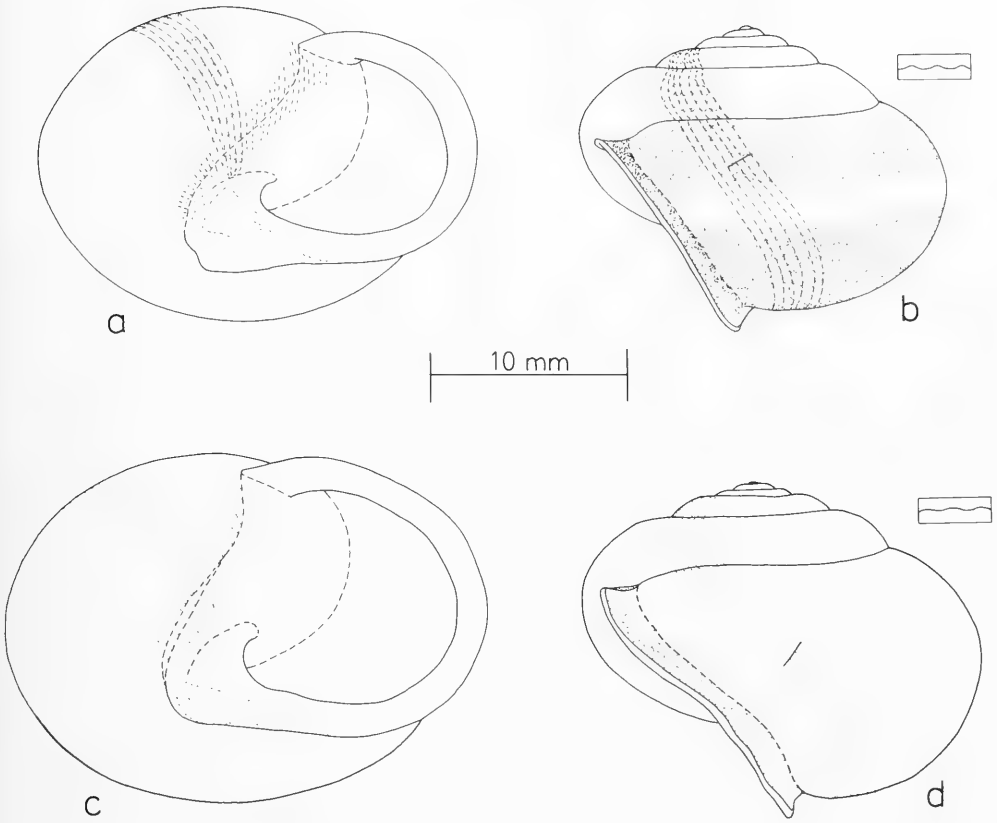

FIG. 32. Shells. **a–b.** *Webbhelix multilineata* (Say, 1821). FMNH 214848 #2. **c–d.** *Neohelix solemi* Emberton, new species. FMNH 214936 #1.

about twice as dense as the columns of wall pustules (8–11 vs. 16–24 for *albolabris albolabris,* and 9–14 vs. 20–22 for *albolabris bogani*), and the same is true of *major* (4–5 vs. 10–16 per 2.6 mm). Since pilastral lappets seem to be derived from wall pustules by lateral fusion (see discussion under Transformation 5), it is assumed that the equal density, or equal width, seen in *alleni* and *solemi* is plesiomorphous. Close examination of Figs. 2d and 11a reveals evidence that the double-sized lappets of *albolabris* and *major* resulted from vertical fusion: one of *albolabris's* lappets has a lateral groove, and two of *major's* lappets have pieces of lappets angled beneath them laterally. This rather obvious character-state transformation was overlooked in the previous analysis.

Transformation D—Preceding transformations: none.

Plesiomorphous state: verge large, greater than .12 the penial length. Present in (outgroups): *W. multilineata* (Fig. 6a), *dentifera* group (Figs. 2a, 5a, d), *albolabris* (Fig. 2d), *major* (Fig. 4a).

Apomorphous state: verge moderate in size, less than .09 the penial length. Formerly and now present in: *alleni* (Fig. 3a, c).

Discussion. The unique, small verge of *solemi,* which is only .01–.05 the penial length (Table 11, column 4) was already used as Transformation 36 (Type 1 small verge). As discussed under that Transformation, the moderately sized terminal verge of *alleni* is not homologous with that of *solemi;* instead, it is structurally (Fig. 3b) very similar to the

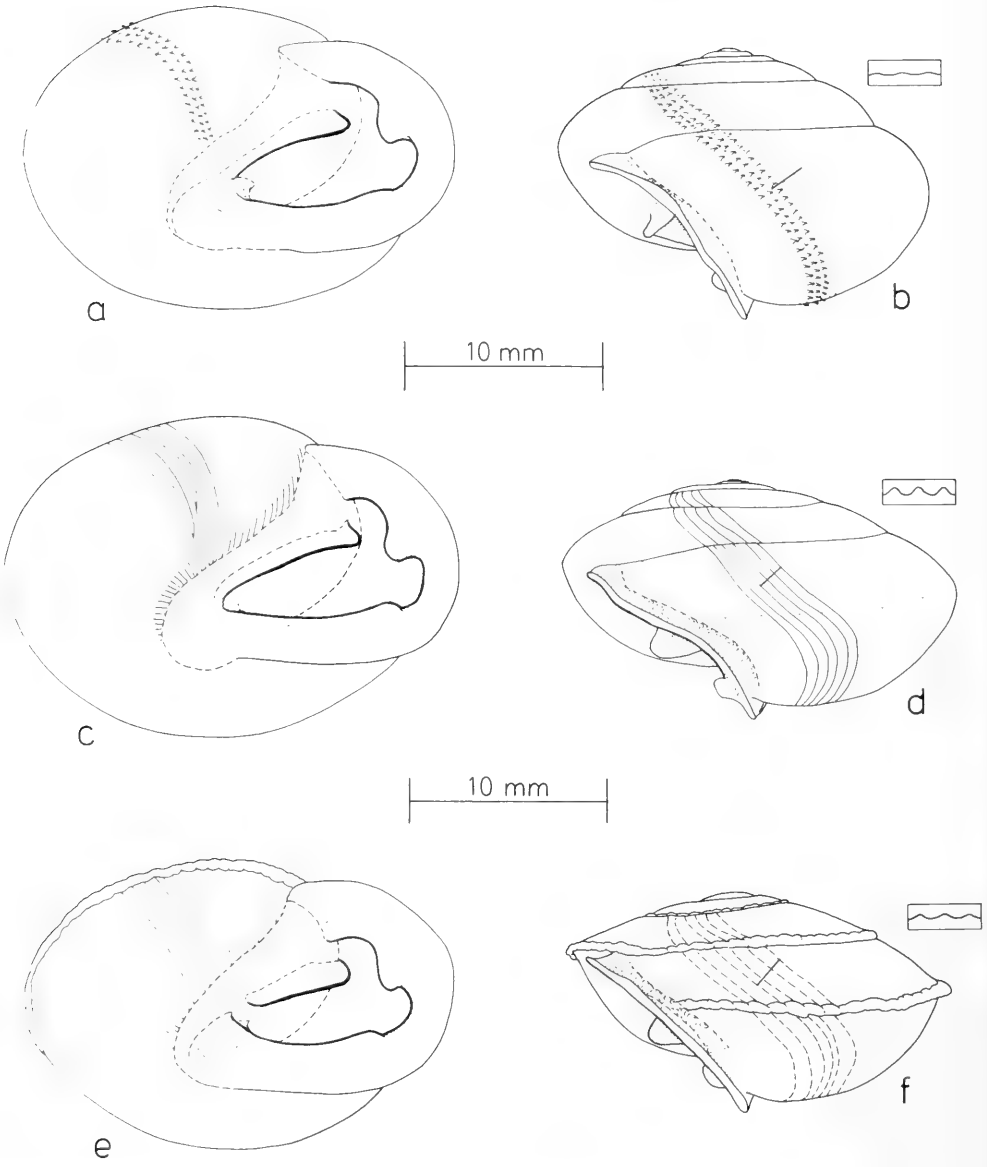

FIG. 33. Shells. **a–b.** *Xolotrema denotata* (Férussac, 1821). FMNH 214806 #1. **c–d.** *Xolotrema caroliniensis* (Lea, 1834). FMNH 171142 #B. **e–f.** *Xolotrema obstricta* (Say, 1821). FMNH 214852 #1.

verges of *albolabris* (Fig. 2f), *major* (Fig. 4b), and *divesta* (Fig. 5e), which differ from it only in their larger size.

Transformation E—Preceding transformations: none.

Plesiomorphous state: pilaster moderate in breadth, .06–.12 the penial length. Present in (outgroups): *W. multilineata* (Fig. 6a), *dentifera* group (Figs. 2a, 5a, d), *albolabris* (Fig. 2d), *major* (Fig. 4a), *alleni* (Fig. 3a, c).

Apomorphous state: pilaster narrow, .02–.04 the penial length. Formerly and now present in: *solemi* (Fig. 6b).

Discussion. The uniquely narrow dorsal pilaster of *solemi* (table 4, column 5) probably

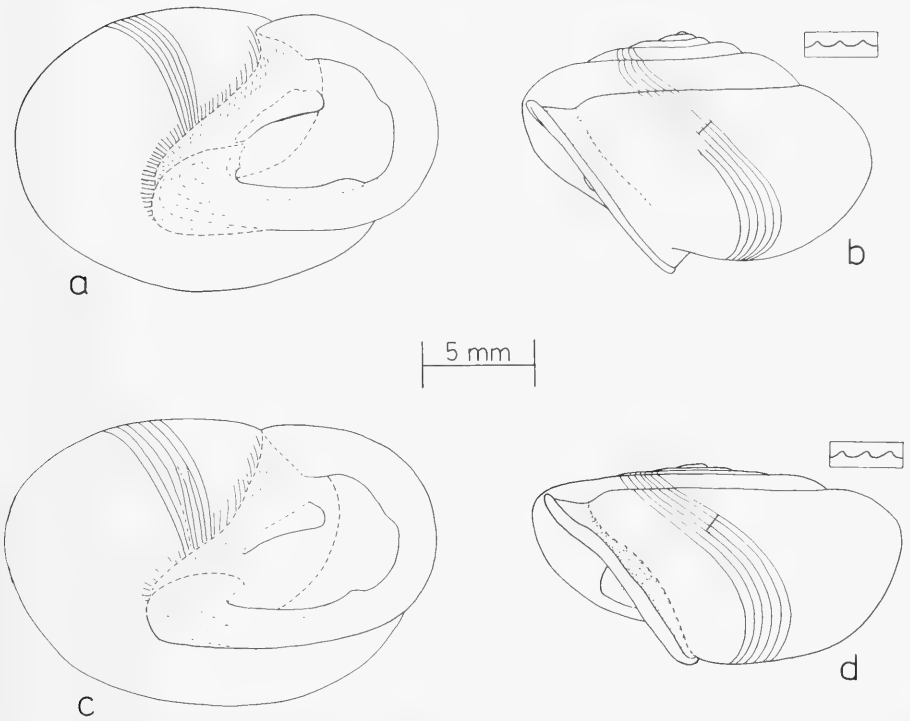

FIG. 34. Shells. **a–b.** *Xolotrema fosteri* (F. C. Baker, 1932). FMNH 214817 #15. **c–d.** *Xolotrema occidentalis* (Pilsbry & Ferriss, 1907). FMNH 214856 #5.

indicates that it is vestigial. The indistinct lappets on this dorsal pilaster (Transformation 6) and the apparently compensatory ventral pilaster (Transformation 44) support this view. It seems likely that when *solemi* evolved a (dorsally) subterminal pore (Transformation 39), the adaptive significance of which is hypothesized in Appendix A, both its verge (Transformation 36) and its dorsal pilaster (Transformation E) were no longer functional and so became vestigial.

Transformation F—Preceding transformations: none.

Plesiomorphous state: retractor muscle's origin distant from the penial apex, .4–.7 the penial length along the vas deferens. Present in (outgroups): *W. multilineata* (Binney, 1851, pl. 8, fig. 2), *dentifera* (Pilsbry, 1940, fig. 491), *divesta* (Pilsbry, 1940, fig. 492; Solem, 1976, fig. 4), *albolabris,* and *major* (Table 4, column 6).

Apomorphous state: retractor muscle's origin close to the penial apex, .1–.3 the penial length along the vas deferens. Formerly and now present in: *alleni, solemi* (Table 4, column 6).

Discussion. In the absence of detailed differences suggesting convergence, it is suggested that this apomorphous character state is homologous in *alleni* and *solemi.*

Transformation G—Preceding transformations: none.

Plesiomorphous state: vas deferens long, over 4 times as long as the penis. Present in (outgroups): *dentifera* (Pilsbry, 1940, fig. 491), (Pilsbry, 1940, fig. 492), *albolabris* (e.g. Pilsbry, 1940, fig. 488) (Table 4, last column), *major* (Table 4, last column).

Apomorphous state: vas deferens short, about 2 times as long as the penis. Formerly and now present in: *alleni, solemi* (Table 4, last column).

Discussion. This hypothesized transformation is somewhat problematic. A short vas deferens occurs in *multilineata* (Binney, 1851, pl. 8, fig. 2), in some *divesta* (Solem, 1976, fig. 4a), and in juvenile *albolabris* (Webb, 1954a, pl. 7, fig. 29); which suggests that the

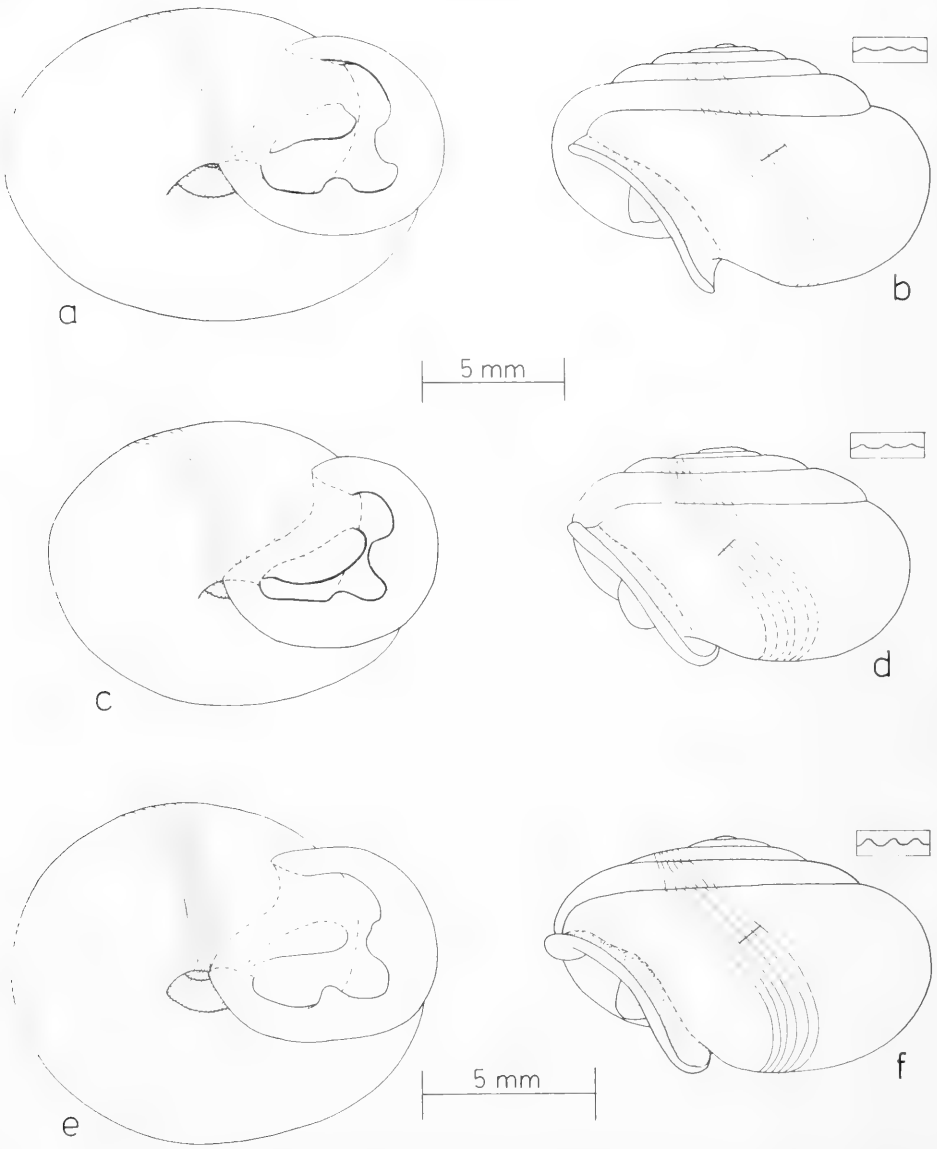

FIG. 35. Shells. **a–b.** *Triodopsis vulgata* Pilsbry, 1940. FMNH 214884 #A. **c–d.** *Triodopsis picea* Hubricht, 1958. FMNH 214860 #15. **e–f.** *Triodopsis claibornensis* Lutz, 1950. FMNH 214800 #A.

long vas deferens of *dentifera, divesta, albolabris,* and *major* may be apomorphous rather than plesiomorphous. However, in keeping with the hypothesis that the *dentifera* group is the immediate outgroup of the *albolabris* group (Fig. 28), outgroup comparison dictates that the short vas deferens of *alleni* and *solemi* is apomorphous. For lack of evidence to the contrary, it is assumed homologous in these two species.

Cladistic analysis

With the addition of Transformations A–G to those used in the Anatomy Tree (Transformations 5–9, 24, 36, 39, 44, 45), there were a total 17 penial-morphological transformations with which to construct a cladogram. These yielded a single, parsimonious cladogram, free of convergence and reversal, which is illustrated in Fig. 48. The only change this

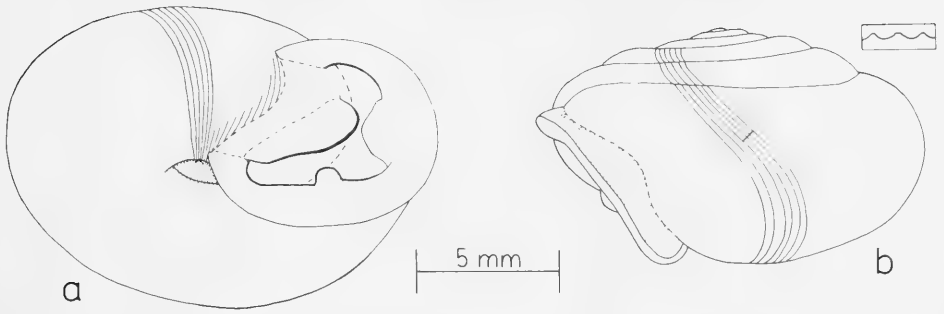

FIG. 36. Shell. **a–b.** *Triodopsis fraudulenta* (Pilsbry, 1894). FMNH 214822 #A.

represents from the Consensus Tree (Fig. 28) is in grouping *alleni* and *solemi* in a monophyletic lineage, designated the *alleni* group.

Shell analysis

The complete shell measurements are presented in Table 6, and are referred to in the systematic review of the *albolabris* and *alleni* groups (Appendix B). The 8 conchological variables, when calculated from the raw measurements (Table 6) by the methods described in Table 5, and when standardized and subjected to discriminant analysis, yielded a discriminant function (Table 7) which correctly classified to subspecies or species 44, or 94%, of the 47 analyzed shells.

The three misclassified shells are marked by asterisks in Table 6. One shell of *alleni alleni* (population 3, specimen #8) was misclassified as *alleni fuscolabris,* with a posterior probability of membership in that subspecies of .66; its probability of correct classification was .34. Two shells of *albolabris albolabris* (population 11, specimen #4; and population 12, specimen #17) were misclassified as *solemi,* with posterior probabilities of membership in that species of .60 and .53 respectively; their probabilities of correct classification were .24 and .47, with the former specimen also having a .16 probability of misclassification in *albolabris bogani.*

Thus, overall, the discriminant function (Table 7) was quite successful in differentiating the 6 taxa by the 8 shell variables (Table 5). The fact that 8 of the 9 total shells of *alleni* were correctly classified to subspecies, and that the ninth shell had a .34 probability of correct classification, is persuasive evidence of the conchological differentiation between the western *alleni alleni* and the disjunct eastern *alleni fuscolabris* (Figs. 46, 50). Likewise, the fact that all of the 21 shells of *albolabris* which were correctly classified to species were also correctly classified to subspecies, testifies to the conchological differentiation between the eastern *albolabris albolabris* and the western *albolabris bogani* (Figs. 47, 49). The discriminant function's marginal failure to differentiate two shells of *albolabris albolabris* from *solemi* points out the necessity of dissection for reliably identifying *albolabris-alleni*-group snails along the northern Piedmont and Coastal Plain (Figs. 47).

Revised classification

The systematic review of the *albolabris* and *alleni* groups is presented in Appendix B. In it, extensive use was made of the tabulated discriminant function (Table 7). Because all 8 variables were standardized (to mean = 0, standard deviation = 1), they received equal weight in the analysis. Therefore, in the discriminant function (Table 7), the total range of a variable is an indication of its value in taxonomically discriminating among the shells. Thus, for example, GLOSSY's range from −10.1 to 8.0 indicates that it is a more powerful discriminator than RELSPIRE, with its smaller range of −1.7 to 1.5. This discriminant function is biased to some degree by the included taxa and the included shells, such that, for example, a reanalysis comparing only *albolabris albolabris* and *albolabris bogani* would produce a different discriminant function emphasizing different variables. In short, Table 7 is not the final or the best word on how to tell these taxa apart by shell characters; it is better viewed as an interim guideline.

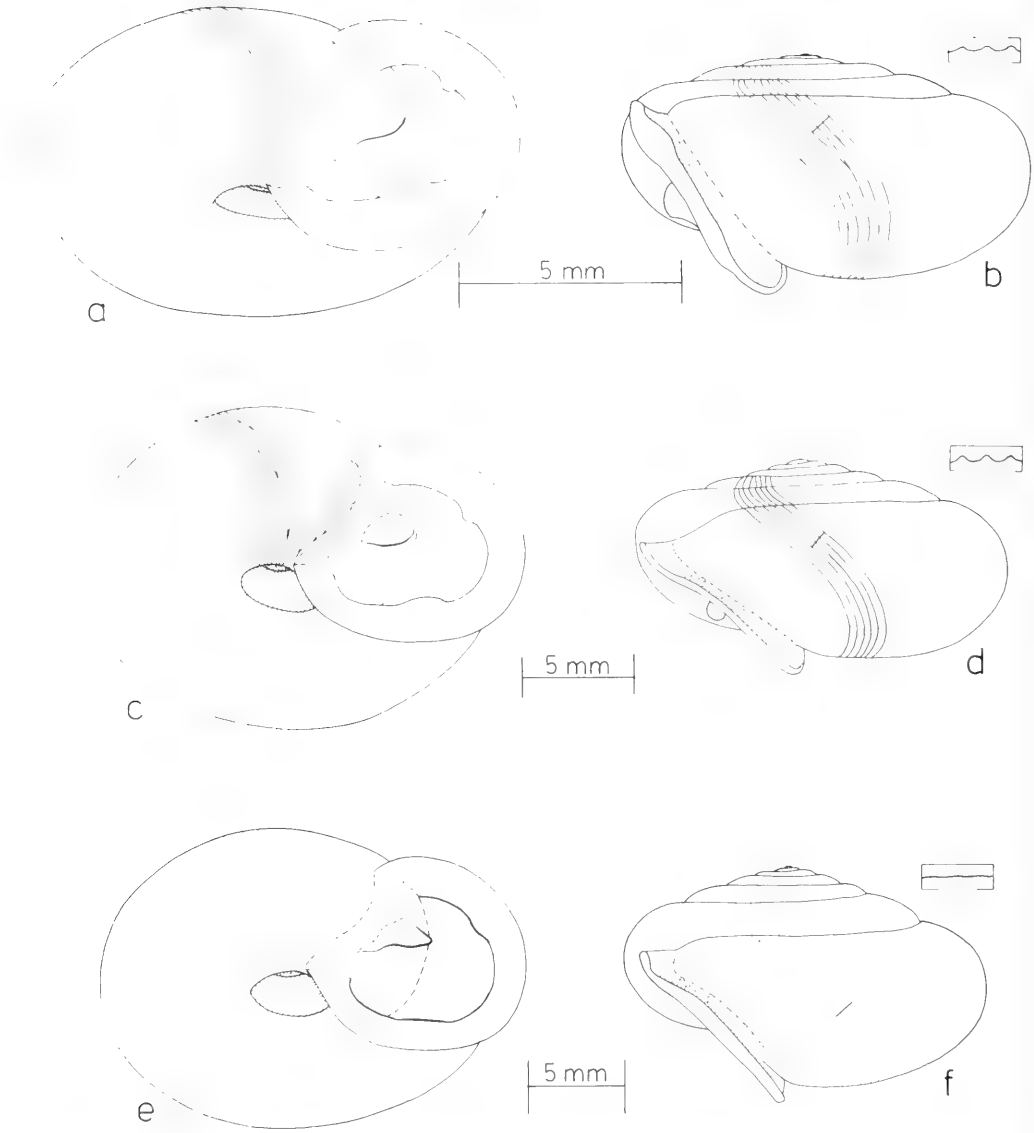

FIG. 37. Shells. **a–b.** *Triodopsis burchi* Hubricht, 1950. FMNH 214797 #10. **c–d.** *Triodopsis tennesseensis* (Walker & Pilsbry, 1902). FMNH 214864 #7. **e–f.** *Triodopsis complanata* (Pilsbry, 1898). Hubricht 17932 #A.

GENERAL SUPRASPECIFIC REVISION

The supraspecific revision of the eastern triodopsines based on the consensus phylogeny (Fig. 28) is listed below and is presented in detail in Appendix C. This revision groups the 40 species into 4 genera, 14 species groups, and 8 species subgroups. Most of the species groups are the same as those temporarily introduced in Table 1 and used throughout the Anatomical and the electrophoretic Trees (Figs. 24–27). Changes from Table 1 are establishment of the *albolabris* and *alleni* groups, expansion of the *vulgata* group to include *fraudulenta* and *picea,* deletion of the *fraudulenta* group, and creation of the *rugosa* group *(rugosa* and *fulciden).* The decision was made reluctantly to submerge

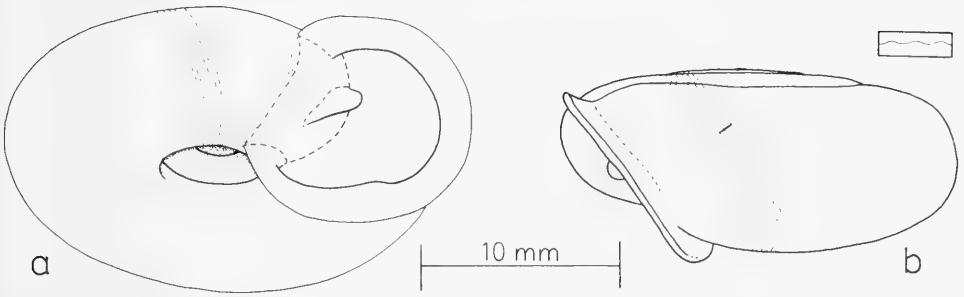

FIG. 38. Shell. **a–b.** *Triodopsis platysayoides* (Brooks, 1933). FMNH 214861 #2.

Webb's subgenera *Wilcoxorbis* Webb, 1952 (= the *fosteri* group) and *Haroldorbis* Webb, 1959 (= the *cragini* group), and section *Shelfordorbis* Webb, 1959 (= the *vulgata* group), because retaining them would have required coining 11 additional subgenera in order to keep the taxonomy hierarchically consistent. The genera and species groups are arranged alphabetically here; in Appendix C they are arranged phylogenetically.

Neohelix von Ihering, 1892
- *albolabris* group
  - *albolabris* (Say, 1816)
  - *major* (Binney, 1837)
- *alleni* group
  - *alleni* (Sampson, 1883)
  - *solemi* Emberton, new species
- *dentifera* group
  - *dentifera* subgroup
    - *dentifera* (Binney, 1837)
  - *divesta* subgroup
    - *divesta* (Gould, 1848)
    - *lioderma* (Pilsbry, 1902)

Triodopsis
- burchi group
  - *burchi* Hubricht, 1950
- *cragini* group
  - *cragini* Call, 1886
  - *henriettae* (Mazÿck, 1877)
  - *vultuosa* (Gould, 1848)
- *fallax* group
  - *alabamensis* subgroup
    - *alabamensis* (Pilsbry, 1902)
    - *hopetonensis* (Shuttleworth, 1852)
    - *vannostrandi* (Bland, 1875)
  - *fallax* subgroup
    - *fallax* (Say, 1825)
    - *messana* Hubricht, 1952
    - *obsoleta* (Pilsbry, 1894)
    - *palustris* Hubricht, 1958
    - *soelneri* (Henderson, 1907)
- *juxtidens* group
  - *juxtidens* subgroup
    - *discoidea* (Pilsbry, 1904)
    - *juxtidens* (Pilsbry, 1894)
  - *neglecta* subgroup
    - *neglecta* (Pilsbry, 1899)
    - *pendula* Hubricht, 1952
- *platysayoides* group
  - *platysayoides* (Brooks, 1933)
- *rugosa* group
  - *fulciden?* Hubricht, 1952
  - *rugosa* Brooks & MacMillan, 1940
- *tennesseensis* group
  - *complanata* (Pilsbry, 1898)
  - *tennesseensis* (Walker & Pilsbry, 1902)
- *tridentata* group
  - *anteridon* (Pilsbry, 1940)
  - *tridentata* (Say, 1816)
- *vulgata* group
  - *fraudulenta* subgroup
    - *fraudulenta* (Pilsbry, 1894)
    - *picea* Hubricht, 1958
  - *vulgata* subgroup
    - *claibornensis* Lutz, 1950
    - *vulgata* Pilsbry, 1940

Webbhelix
- multilineata (Say, 1821)

Xolotrema
- denotata group
  - *denotata* (Férussac, 1821)
  - *caroliniensis* (Lea, 1834)
  - *obstricta* (Say, 1821)
- *fosteri* group
  - *fosteri* (F. C. Baker, 1932)
  - *occidentalis* (Pilsbry & Ferris, 1907)

Table 8 compares this classification with those of Pilsbry (1940) based on shell morphology; Webb (1952, 1954, 1959), based on

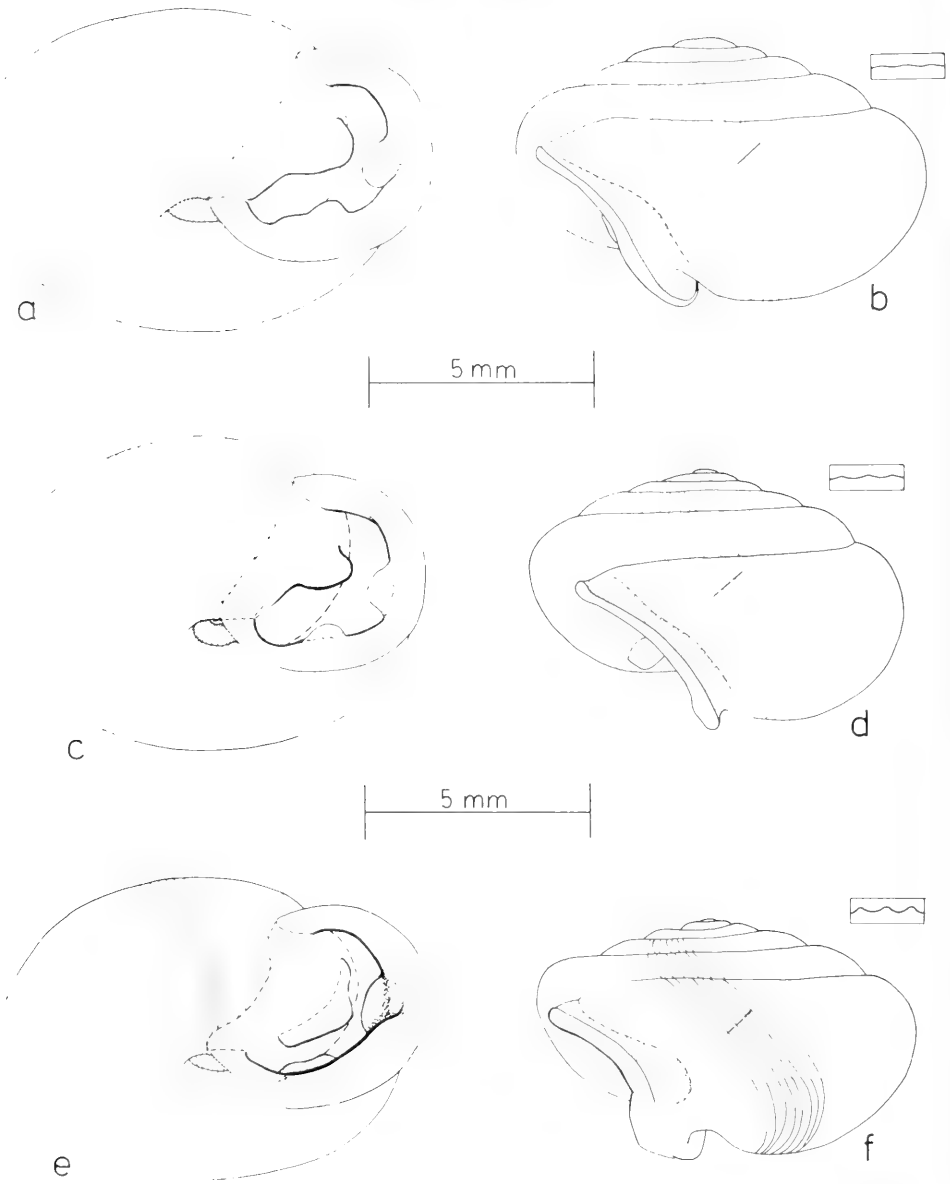

FIG. 39. Shells. **a–b.** *Triodopsis vultuosa* (Gould, 1848). FMNH 214887 #7. **c–d.** *Triodopsis cragini* Call, 1886. FMNH 214803 #2. **e–f.** *Triodopsis henriettae* (Mazÿck, 1877). FMNH 214824 #2.

reproductive anatomy and behavior; and Vagvolgyi (1968), based on shell morphology. Of the 40 species recognized here, Pilsbry classified 33, Webb 15, and Vagvolgyi 38. Irrelevant of the number of species, this revision most closely resembles the classification of Pilsbry (1940) as modified by Hubricht (1985)—the major difference lies in the grouping of species within *Triodopsis* (Table 8).

The systematics of the *Triodopsis fallax* group presented in Appendix C and Table 9 is that of Grimm (1976), as discussed in Appen-

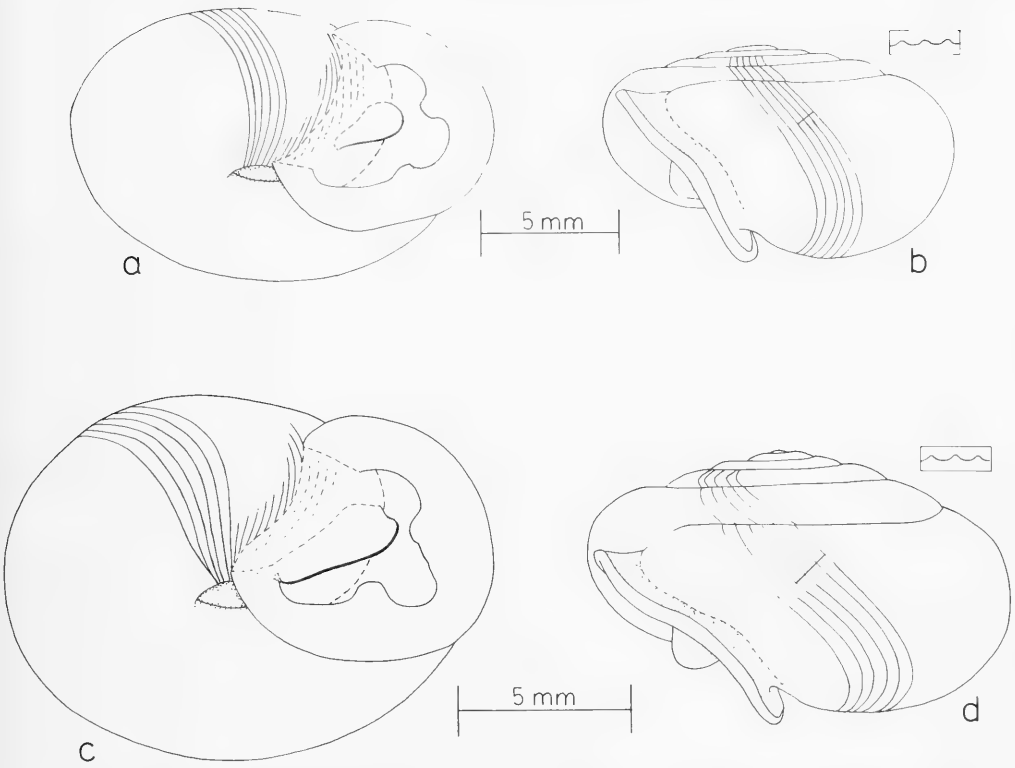

FIG. 40. Shells. **a–b.** *Triodopsis tridentata* (Say, 1816). FMNH 214876 #4. **c–d.** *Triodopsis anteridon* (Pilsbry, 1940). FMNH 214796 #19.

dix D. Division of the *juxtidens* group into *juxtidens* and *neglecta* subgroups is based on shell morphology—see Appendix C.

PATTERNS OF GENITALIC EVOLUTION

The ranges of the 40 species of eastern triodopsines are presented in Fig. 49. These maps were compiled from Hubricht (1985), with corrections for the *Neohelix albolabris* and *alleni* groups.

The maps were used to compare the degree of difference in penial morphology of sister taxa with their geographic range relationship. The results based on 25 comparisons (Table 9) are: sister taxa with virtually identical penes generally have peripatric ranges, those slightly different are generally allopatric, those moderately different are sympatric, but those greatly different are parapatric.

The tests for population-level reproductive character displacement are summarized in Table 10. In none of these 12 tests was there any detectable difference in penial morphology between allopatric and sympatric populations.

PATTERNS OF SHELL EVOLUTION

Fig. 50 shows the phylogenetic pattern of shell morphology among all known living species of eastern North American triodopsines. A general evolutionary pattern is of conchological stasis within genera. In general, each genus is characterized by a distinct shell form: *Neohelix* and *Webbhelix* shells are large, globose, and toothless (Figs. 29–32); *Xolotrema* shells are medium-sized and depressed, with a blade-like parietal tooth and a long basal lamella (Figs. 33, 34); and *Triodopsis* shells are small, subglobose, and tridentate (Figs. 35–45).

Shell convergences among these conchologically distinct genera are rare. *Webbhelix* and *Neohelix* shells are similar appar-

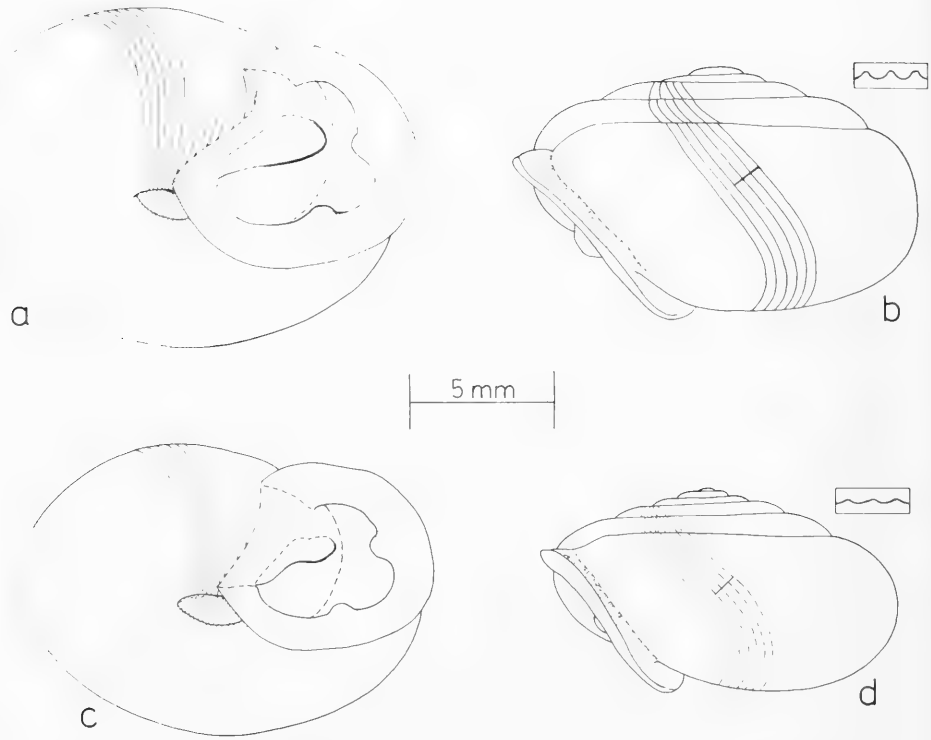

FIG. 41. Shells. **a–b.** *Triodopsis juxtidens* (Pilsbry, 1894). FMNH 214841 #7. **c–d.** *Triodopsis discoidea* (Pilsbry, 1904). FMNH 214811 #A.

ently because they share the plesiomorphous shell morphology seen in some of their outgroups (Fig. 50). One *Neohelix* species—*dentifera* (Fig. 29a–b)—converged slightly on *Xolotrema* by its low spire, strong parietal tooth, and suggestion of a basal lamella. Two lineages of *Triodopsis—platysayoides* (Fig. 38) and *tennesseensis* group (Fig. 37c–f)—converged, apparently independently, on *Xolotrema* by evolving enlarged, depressed shells with reduced outer lip teeth. These convergences are not very close, hence the shells are easily assigned to the correct genus.

Within a genus, the distributional pattern of shell characters among species groups and among species is generally mosaic, with many cases of convergence or parallelism. Within *Neohelix,* a parietal tooth crops up in both the *albolabris* group (some *albolabris* populations—see Pilsbry, 1940) and the *dentifera* group (*dentifera,* Fig. 29a); a basocolumellar lip node appears in both the *albolabris* group (*major,* Fig. 30c) and the *alleni* group (*alleni,* Fig. 30a); and a glossy yellowish periostracum arises in all three species groups *(albolabris hubrichti, alleni alleni,* and *lioderma).* Within *Xolotrema,* a carinate shell occurs convergently in both the *fosteri* group (*occidentalis,* Fig. 34d) and the *denotata* group (*obstricta,* Fig. 33f). Within *Triodopsis,* enlarged, flat shells with weak dentition appear in both *platysayoides* (Fig. 38) and the *tennesseensis* group (Fig. 37c–f); a squared-off parietal tooth occurs independently in the three species-groups *vulgata* (Figs. 35a, c, e, 36a), *rugosa* (Fig. 45c-), and *juxtidens* (Figs. 41c, 45a, e); a buttressed palatal tooth of identical appearance shows up in both *rugosa* of the *rugosa* group (not illustrated) and *anteridon* of the *tridentata* group (Fig. 40c); and toothless apertural lips occur convergently in three species in three disparate lineages: *platysayoides* (Fig. 38a), *soelneri* (Fig. 44c), and in rare populations of *tridentata* (see Pilsbry, 1940); a glossy periostracum appears in the *tennesseensis* group (*complanata,* Fig. 37f) and twice, ap-

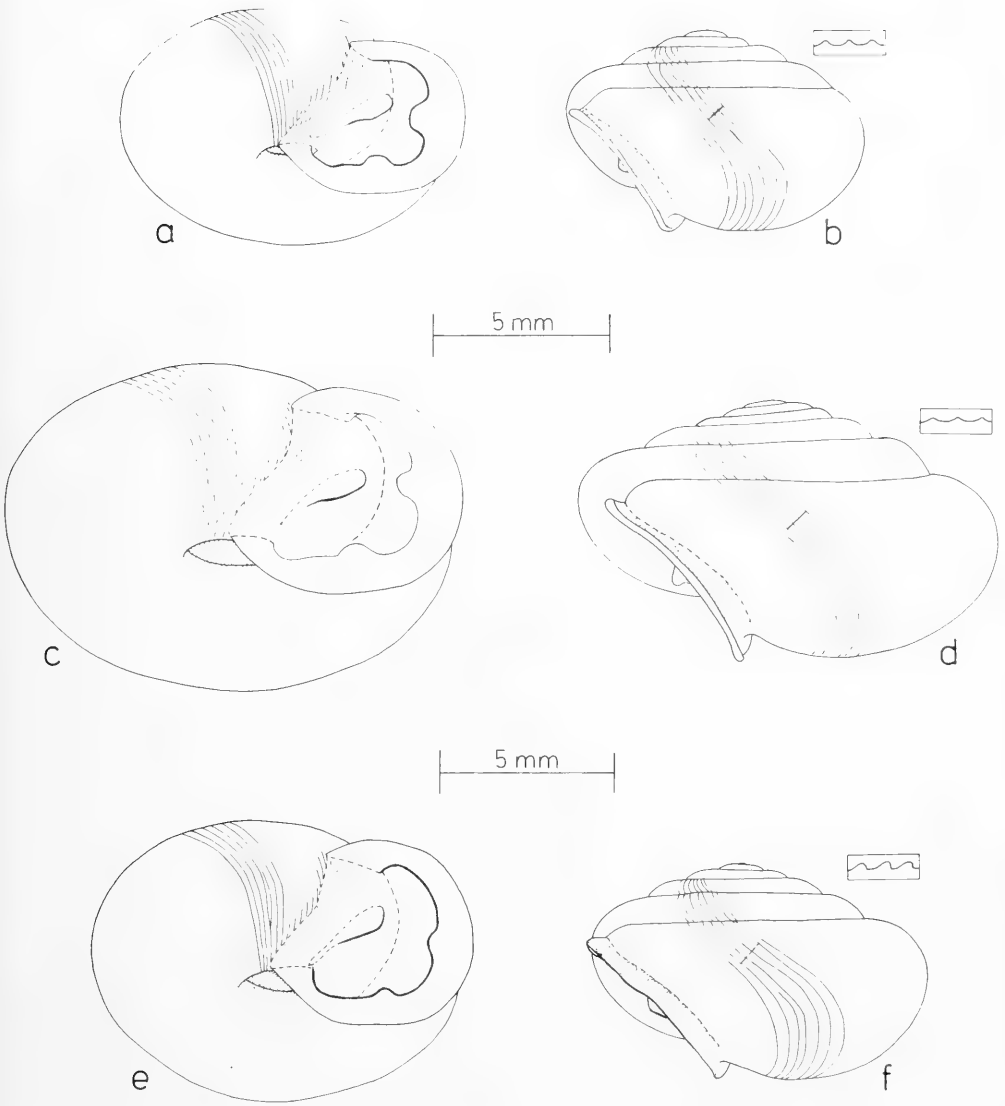

FIG. 42. Shells. **a–b.** *Triodopsis hopetonensis* (Shuttleworth, 1852). FMNH 214827 #22. **c–d.** *Triodopsis palustris* Hubricht, 1958. FMNH 214857 #1. **e–f.** *Triodopsis obsoleta* (Pilsbry, 1894). Hubricht 10300 #A.

parently independently, in the *fallax* subgroup (*palustris,* Fig. 42d; and *soelneri,* Fig. 44d). Other examples of intrageneric shell convergences among species groups and species could be cited, but these are the most conspicuous.

Shell variation within species is summarized in Table 11, which compiles Vagvolgyi's (1968) data with taxonomic corrections. Shell size, spire height, umbilical relative width, and whorl count vary greatly. Diameter range covaried significantly with sample size, whether expressed as number of lots ($r = 0.67$, d.f. = 23) or as total number of shells ($r = 0.64$, d.f. = 23). For wide-ranging, well-sampled species from all four genera (e.g., *W. multilineata, N. albolabris, X. fosteri,* and *T. tridentata*), diameter ranged approximately 70%. Maximum diameter range was found in *juxtidens:* 95%.

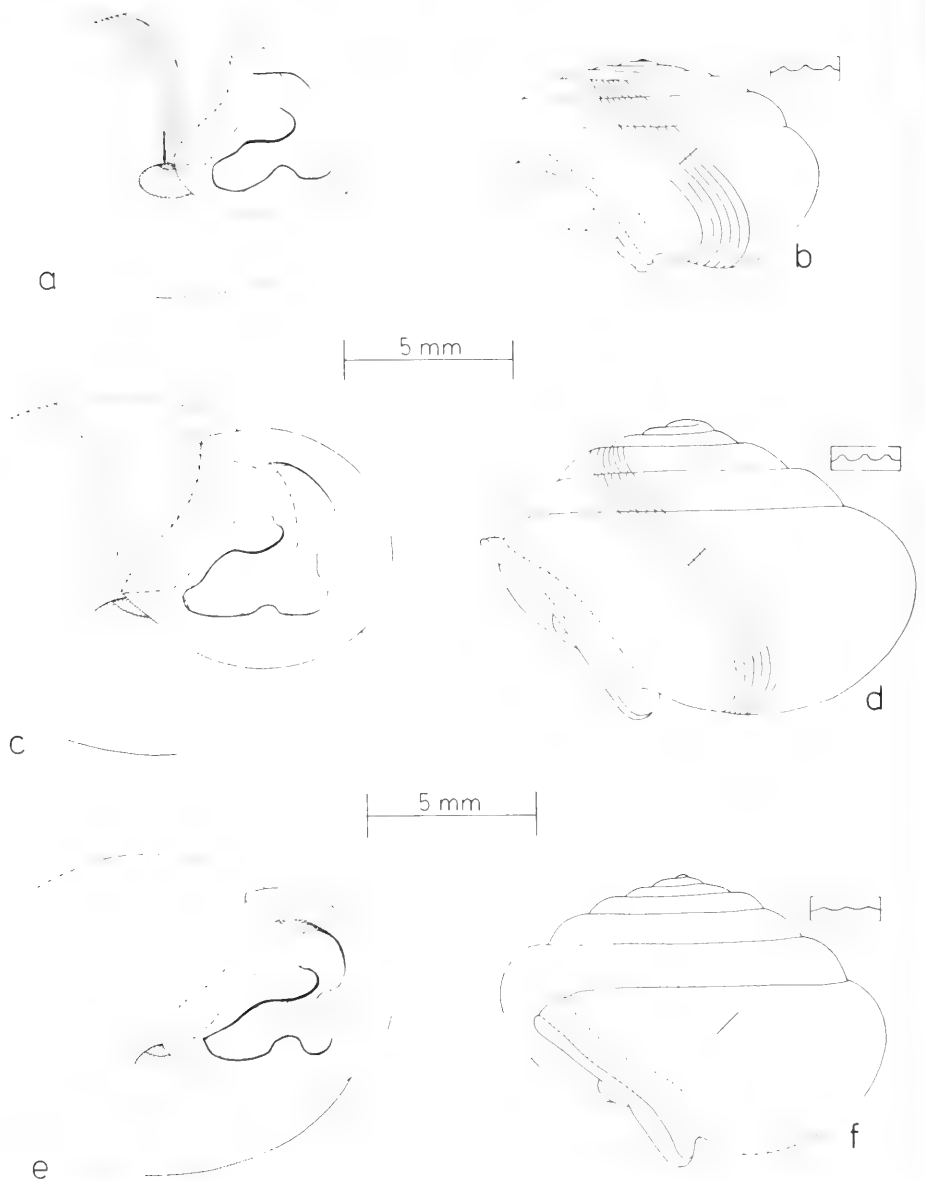

FIG. 43. Shells. **a–b.** *Triodopsis alabamensis* (Pilsbry, 1902). FMNH 214791 #A. **c–d.** *Triodopsis messana* Hubricht, 1952. FMNH 214846 #A. **e–f.** *Triodopsis vannostrandi* (Bland, 1875). FMNH 214880 #11.

DISCUSSION

Genitalic analysis

The penis proved to be an outstanding tool for the erection of a cladistic hypothesis for the eastern triodopsines. Its morphological diversity yielded an unprecedented number (for pulmonates) of character states, and its sculptural complexity permitted the detection of many convergences.

The suggested character-state transformations (Figs. 19–23) varied considerably in plausibility. The thoroughness of their documentation, however, establishes an objective baseline for future, more enlightened revisions.

The choice of PAUP (Swofford, 1983) for

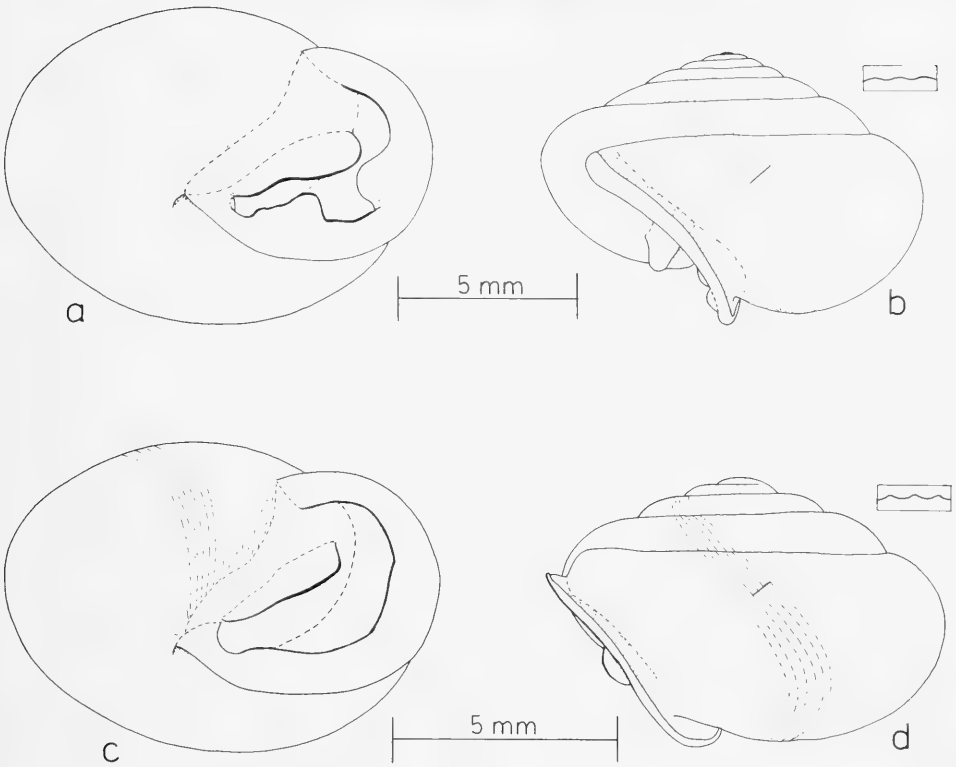

FIG. 44. Shells. **a–b.** *Triodopsis fallax* (Say, 1825). Hubricht 10209 #A. **c–d.** *Triodopsis soelneri* (Henderson, 1907). FMNH 159040 #A.

constructing cladograms has recently received support by Fink's (1986) comparisons of available software: PAUP was clearly more reliable than PHYLIP for finding the shortest trees. The Anatomy Tree generated from the triodopsine data by PAUP (Fig. 24) is remarkable for its high consistency index and for its uniqueness as the single most parsimonious cladogram. See Fink (1986) for an introduction to alternatives to the maximum-parsimony approach to cladogram construction used here.

Allozymic analysis

The number of snails electrophoresed per population averaged 3.9 (standard deviation 2.5). Although larger sample sizes would certainly have been preferred, small samples are generally sufficient for detecting systematic affinity from allozymes (Sarich, 1977; Gorman & Renzi, 1979; Shaffer, 1984; also compare the "exemplar method" of Sokal & Sneath, 1963).

Buth (1984) evaluated the available methods for applying electrophoretic data to systematics studies. Of his concluding list of 8 recommendations—(1) sample intraspecific geographic variation, (2) list raw data, (3) code allozyme data with the locus as the character for cladistic analysis, but also consider distance methods, (4) state the procedure used for ordering the transformations used for cladistic analysis, (5) construct minimum-length Wagner trees for cladistic analysis, because of their freedom from the assumption of constant evolutionary rates, (6) use outgroup comparison to determine the polarities of transformations, (7) check homoplasious steps in the constructed cladogram for possible introgressive origins, and (8) separately and identically analyze two independent data sets and examine them for congruency—all but numbers 3 and 7 were followed in this paper. Instead of Buth's number 3 recommendation to code loci as the cladistic characters, individual alleles were coded, thereby using the "independent allele model" introduced by Mickevich & Johnson (1976), "[treating] each allele as a

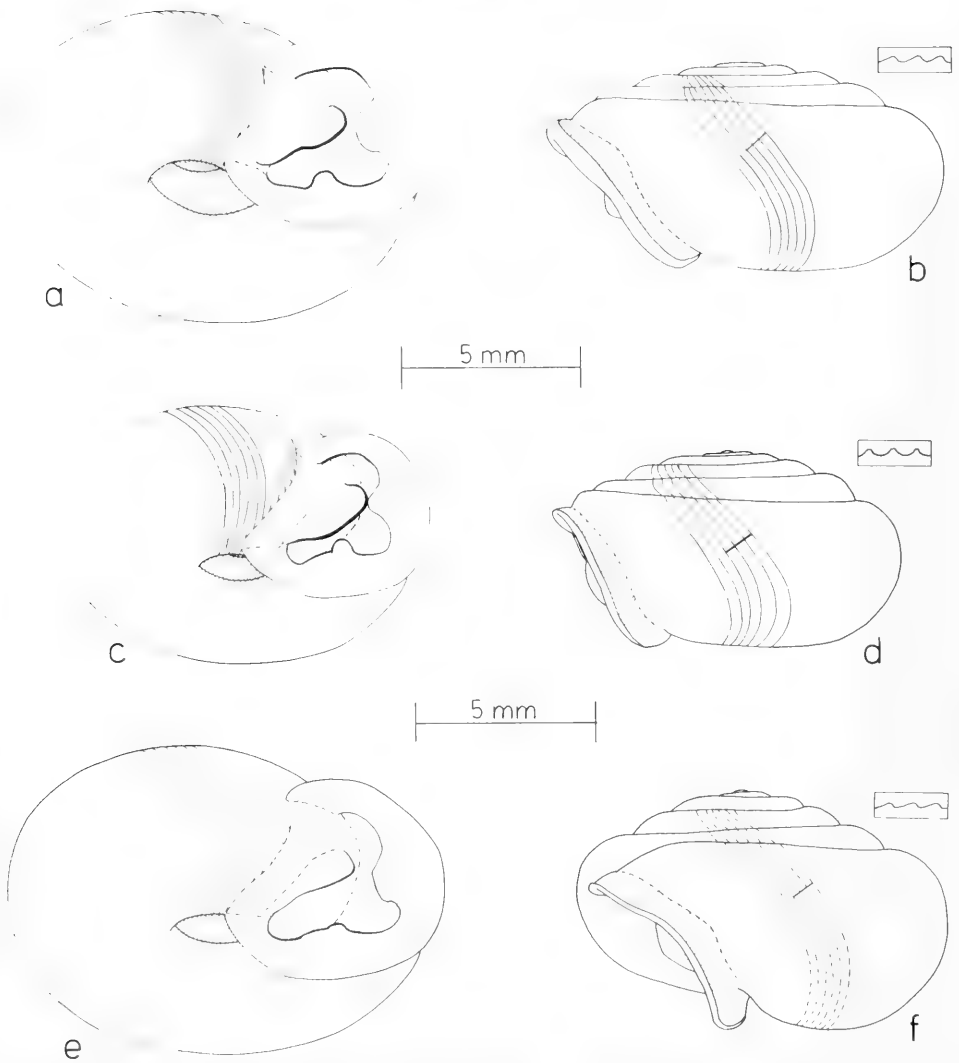

FIG. 45. Shells. **a–b.** *Triodopsis neglecta* (Pilsbry, 1899). FMNH 214850 #A. **c–d.** *Triodopsis fulciden* Hubricht, 1952. FMNH 214823 #A. **e–f.** *Triodopsis pendula* Hubricht, 1952. FMNH 214859 #14.

binary character to be scored merely as present or absent" (Mickevich & Mitter, 1981). This scoring method has the disadvantages—probably minor—of occasionally being biologically unrealistic by hypothesizing intermediates which lack alleles at a locus and by making the assumption that alleles are indeed always independent (Mickevich & Mitter, 1981). These disadvantages of the independent allele model are outweighed by its advantage of producing unquestionably ordered transformations (present/absent)—in this it differs importantly from coding the locus as the character, for which method "the problem of ordering is currently the most critical [unsolved] issue in this research area" (Buth, 1984). Of the several systems for coding independent alleles the present/absent system used in this paper is the method of choice "[when] the cladistic informativeness of frequency changes is suspect or demonstrably small" (Mickevich & Mitter, 1981), both of which conditions apply to the eastern-triodopsine data set (Table 2). Buth's number 7 recommendation to check for introgressive origins of homoplasies was not feasible for the triodopsine data set because the degree of interspecific hybridization

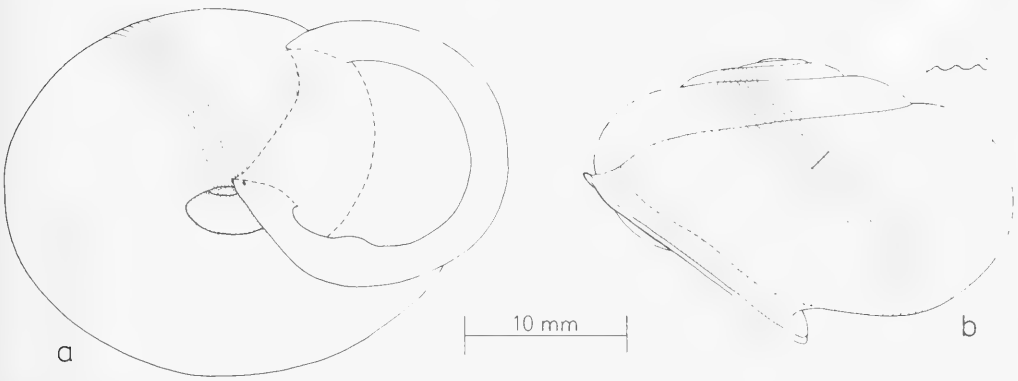

FIG. 46. *Allogona profunda* (Say, 1821). FMNH Uncat. #3. **a–b.** Shell.

TABLE 4. Measurements of penial morphology for the 6 species and subspecies of the *Neohelix albolabris* and *alleni* groups, expressed as ranges over three measured dissections (one per population). Underlined ranges are those disjunctly different from *albolabris*.

Species or subspecies	Penis length (mm)[1]	No. pilastral lappets per 2.6 mm	No. columns of pustules per 1.3 mm[2]	Verge length: penis length	Pilaster breadth: penis length	Retractor M. distance from penis apex: penis length	Vas deferens length:[3] penis length
alleni plus *fuscolabris*	10–18	15–18	9–11	.08–.09	.09–.12	.1–.3	2.1–2.4
albolabris	10–16	8–11	8–12	.15–.21	.06–.12	.4–.7	5.1–5.6
bogani	10–13	9–14	10–1	.14–.16	.07–.11	.4–.7	4.6–5.7
major	17	4–5	5–8	.12–.15	.08–.11	.4–.5	4.2–4.5
solemi	12–17	14–15	4–6	.01–.05	.02–.04	.1–.3	1.8–2.5

[1]From junction with atrium to *internal* apex.
[2]Adjacent to pilaster about two-thirds the distance from penial internal apex.
[3]From 'Y' of the atrium to insertion at penial apex.

within this group of snails is very poorly known.

The second part of Buth's (1984) number-3 recommendation to "[consider] the interpretation of distance treatments Felsenstein (1984) advanced" was followed by transforming the electrophoretic data into genetic distances, then applying a clustering algorithm. The advantages of the Prevosti genetic distance coefficient used in this analysis are its simplicity and its 0-to-1 range; the "[single] theoretical objection . . . [that it] gives equal weight to frequency differences throughout the range from 0 to 1" (Wright, 1978) does not seem critical for this data set, in which allelic frequencies can vary greatly within a species, in which heterozygosity is extremely low, and in which species usually differ by fixed, alternative alleles rather than by frequency differences among the same alleles (Table 2). The Prevosti coefficient was chosen over two which predominate in the literature—those of Nei (1972, 1978) and Rogers (1972). The failure of Nei's distance coefficient to satisfy the triangle inequality (Farris, 1981), although probably not critical theoretically (Felsenstein, 1984), can produce the practical disadvantage of negative branch lengths, "an undesirable and biologically uninterpretable result for a coefficient used in reconstructing phylogenies" (Buth, 1984). Roger's distance coefficient satisfies the triangle inequality (Buth, 1984), but has the disadvantage of being "a mixed concept depending on the degree of [allelic] fixation as well as degree of difference in such a way that two populations with fixa-

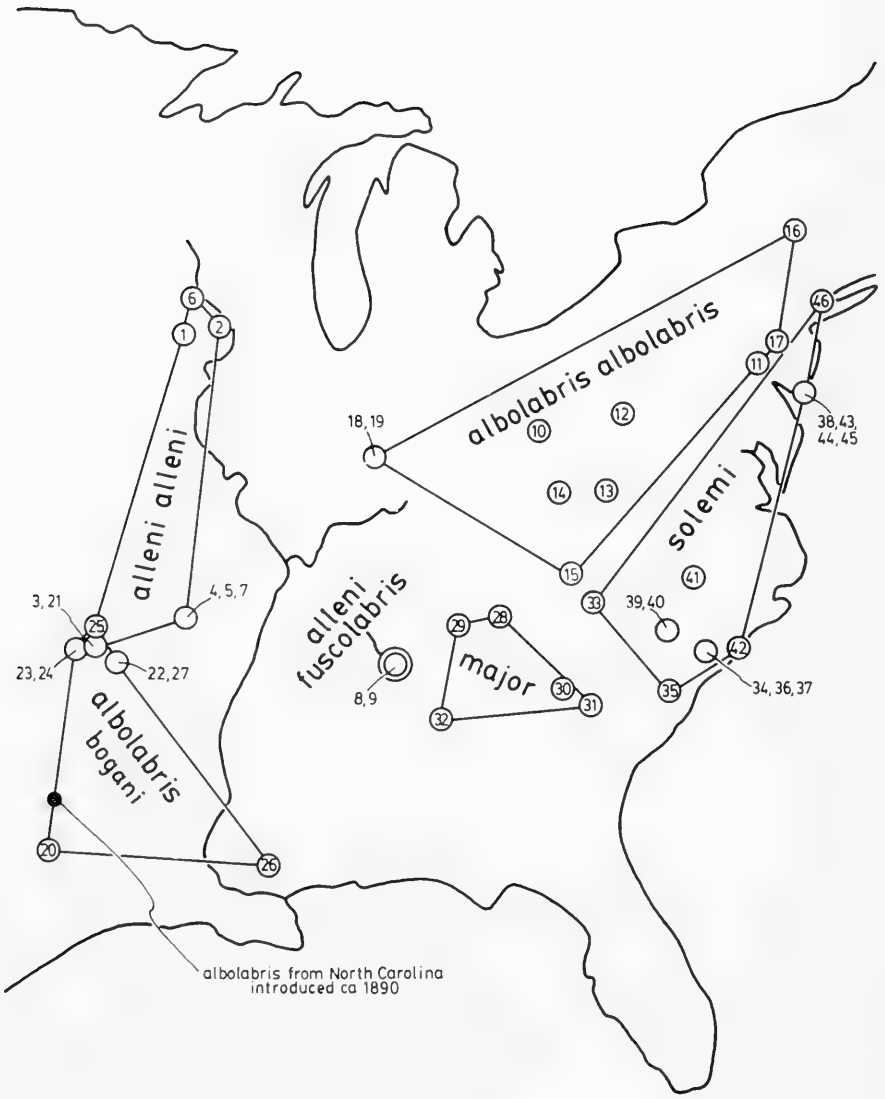

FIG. 47. Geographical distribution of the 46 populations studied for the revision of the *Neohelix albolabris* group.

tion of different alleles are considered farther apart than ones where both are heterallelic even though they have no common allele" (Wright, 1978). In retrospect, a better choice than the Prevosti coefficient would have been the Cavalli-Sforza & Edwards (1967) chord measure advocated by Wright (1978) and Felsenstein (1984).

For clustering taxa from genetic distance data, three algorithms are most commonly used: UPGMA (unweighted pair-group with arithmetic averaging: Sokal & Sneath, 1963;

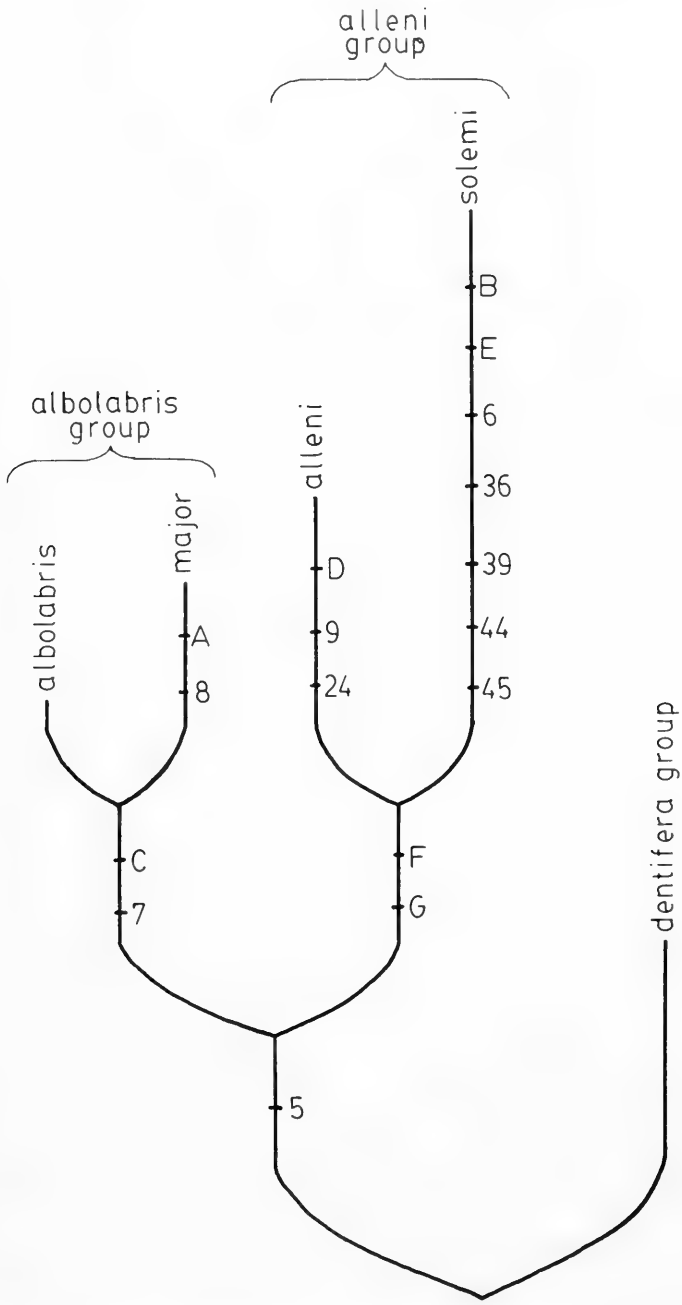

FIG. 48. Revised phylogenetic hypothesis for the *Neohelix albolabris* group, based on the addition of penial-morphological transformations A–G, with the *dentifera* group as closest outgroup. This cladogram justifies splitting the *albolabris* group into the *albolabris* and *alleni* groups.

Sneath & Sokal, 1973; Nei, 1978), Fitch-Margoliash (Fitch & Margoliash, 1967), and distance-Wagner (Farris, 1972). For this paper, the distance-Wagner procedure was chosen because it provides the best fit to the original distance data, it is free from the

TABLE 5. Shell variables used in discriminant analysis (Table 7) among the 6 species and subspecies of the *Neohelix albolabris* and *alleni* groups.

Variable	Abbreviation	Method of measurement or calculation (from Table 6)
Striae per 2.6 mm	STRIAE	Striae count (number of striae per 2.6 mm on upper surface of the end of the fifth whorl).
Brownness	BROWN	Color rank (1 to 7, ranging from light yellow to dark brown).
Glossiness	GLOSSY	Sheen rank (1 to 6, ranging from glossy to dull).
Relative height of spire	RELSPIRE	Shell height divided by shell diameter.
Whorl expansion rate	WHRLEXPN	Shell diameter divided by whorl number.
Relative size of baso-columellar node	RELNODE	Width of apertural lip at midnode, divided by width of apertural lip at its narrowest basal point.
Relative width of apertural lip	RELLIP	Width of apertural lip at periphery, divided by shell diameter.
Relative pre-apertural deflection of body whorl	RELDEFL	Body-whorl depth behind lip, minus pre-deflection body-whorl depth, divided by shell diameter.

assumption of constant evolutionary rates (unlike UPGMA and other agglomerative methods, and unlike the "Fitch-Margoliash" method of Prager & Wilson, 1978), and it is computationally feasible (unlike the true Fitch-Margoliash method) (Farris, 1981; Swofford, 1981; Tateno et al., 1982; review in Buth, 1984). Although Farris (1981) later repudiated his own distance-Wagner method (Farris, 1972) and all other methods of inferring phylogenies from distances, as inherently unable to reconstruct branch lengths consistent with evolutionary events, Felsenstein (1984) showed that "[Farris's] major criticisms of these methods lose their force" when an alternative, statistical (rather than absolute) interpretation of branch lengths is used. A drawback of the distance-Wagner algorithm is that the final tree topology is to some extent dependent on the order in which the distance data are read in for computation (e.g., Swofford & Selander, 1981). This drawback could have been (but was not for this paper) corrected by using rearrangement algorithms which shuffle and refeed the data, but even without such safeguards, "the distance-Wagner procedure is likely to be much more effective than [the Fitch-Margoliash procedure]" (Swofford, 1981).

Robustness of the consensus phylogeny

Anatomical and electrophoretic data sets were remarkably congruent. The final Consensus Tree (Figs. 28, 50) is virtually identical to the Anatomy Tree (Fig. 24), requiring little modification to comply with the electrophoretic trees (Figs. 25–27).

Thus the electrophoretic data validated the cladistic analysis of penial morphology. There is slight circularity in this statement, because *Triodopsis fraudulenta* and *T. neglecta* were anatomically reevaluated due to discrepancies between electrophoretic and anatomical data. This circularity is trivial in the context of the entire phylogeny, but it vouches strongly for the importance of comparing independent data sets to guard against misinterpretations.

Genitalic evolution: pattern and process

The results of 25 comparisons between sister taxa, presented in Table 9, showed a counter-intuitive trend. Sister taxa with virtually identical penial morphologies generally have peripatric ranges, those slightly different are generally allopatric, those moderately different are sympatric, but those greatly different are parapatric.

Several shortcomings of the data need to be considered before interpreting this result. First, two of the taxon pairs are questionable (and are so marked in Table 9), because of inadequate data or a discrepancy between anatomical and electrophoretic data. Second, those ranges were called parapatric or peripatric which appear in Fig. 49 to be contiguous or only slightly overlapping, without an intervening geographical barrier; because these maps are imprecise, some of interpretations may be incorrect. Third, current range relationships may have little relevance to those under which the anatomical changes actually evolved, because of distortion by Plio-Pleistocene and perhaps earlier climatic and vegetational changes.

If one assumes correct phylogeny and correct interpretation of temporally stable ranges, then four hypotheses concerning evolutionary processes can be proposed. (1) Peripheral isolates generally do not differentiate. (2) Vicariant isolates generally differentiate slowly. (3) Differentiation due to reproductive character displacement is moderate at most. (4) Extreme differentiation is rare, rapid, and occurs in isolates. Each of these will be discussed in turn.

Peripheral isolates generally do not appear to differentiate, because all 8 examples of peripatric sister taxa have identical genitalia (Table 9). This is consistent with the overall lack of intraspecific geographic variation found in the eastern triodopsines, as mentioned previously. There may be some natural-selected inertia to change in genital morphology due to founder effects or population bottlenecks, because these events are probably common in triodopsine species. For example, populations of *Neohelix albolabris* are patchily distributed and ephemeral, with draught and predation periodically producing local die-backs or extinctions, followed during favorable years by rapid build-ups from survivors or founders (McCracken, 1976). There would be a selective advantage to groups of *N. albolabris* in which flush-crash populations conserved ancestral genitalic morphology and were thus able to restore their genetic diversity by remating with other populations during flushes. Indeed the penial morphology of this species is remarkably uniform over its very wide geographic range (Fig. 47, Table 4).

Vicariants generally appear to differentiate slowly, because all four examples of allopatric sister taxa differ only slightly in their genitalia (Table 9). This hypothesis is further supported by two species of *Neohelix (albolabris* and *alleni),* both of which have two subspecies that have been genetically isolated by the Mississippi River (Figs. 47, 49) for at least 20,000 years (see Delcourt & Delcourt, 1981)—equivalent to at least 10,000 generations (McCracken, 1976)—and that have evolved significant shell differences (Table 7), yet are virtually identical in penial sculpture (Fig. 3, Table 4).

Differentiation due to character displacement appears to be moderate at most, because all 5 examples of sympatric sister taxa had only moderate genitalic differences, and because none of the 6 examples of sister taxa showing greater than moderate differences were sympatric. All 5 sympatric pairs are probably microsympatric. In three of them (*T. soelneri* vs. both *T. messana* and *T. hopetonensis; T. tridentata* vs. *T. juxtidens;* and *N. albolabris* vs. both *N. dentifera* and *N. divesta*) the taxa have been found within crawling distance of each other with no evidence of hybridization (personal observation); it is likely that the other two examples *(N. albolabris bogani* vs. *N. alleni alleni,* and *W. multilineata* vs. *N. albolabris)* also come into contact, with no hybrids known. In all of these cases, the penial differences were primarily in the dorsal pilaster, with occasional differences in the wall pustulation as well. These differences may be sufficient in themselves for mate recognition, but there other possible isolation mechanisms that prevent sister-species hybridization and hence that diminish the role of reproductive character displacement in causing morphological divergence. Despite Webb's (1948, 1952, 1959, 1961) conclusion that penial sculpture is the basis of mate-recognition in triodopsines, interspecific matings do occur under laboratory conditions (Grimm, 1975), even between such genitalically different species *T. tridentata* and *T. vulgata* (Webb, 1948). Thus pheromones, courtship behavior, and post-mating isolating mechanisms may also play some role in mate recognition. In addition, in some of these cases of sympatric sister species, there are varying degrees of habitat difference, suggesting that ecological character displacement limits reproductive contact. Both *W. multilineata* and *T. soelneri* inhabit marshier habitats than their sister taxa (e.g., Vagvolgyi, 1968; Hubricht 1985); *N. alleni fuscolabris* inhabits a more alkaline, limestone habitat than *N. major* (Hubricht, personal communication; personal observations); and *T. discoidea* is found on river bluffs, whereas *T. tridentata* is found in woods above the bluffs (Vagvolgyi, 1968; personal observations). No conspicuous habitat differences which would restrict contact are known for the four pairs N. alleni alleni vs. *N. albolabris hubrichti, N. divesta* vs. *N. albolabris hubrichti, N. dentifera* vs. *N. albolabris albolabris,* or *T. tridentata* vs. *T. juxtidens.* The first two of these pairs need investigation (see Solem, 1976); the third is currently under study in Virginia by T. Asami; and the fourth shows a mozaic distributional pattern (Pilsbry, 1940) suggestive of competition, although they are occasionally found microsympatric (Vagvolgyi, 1968; personal observation).

TABLE 6. Shell measurements of the *Neohelix albolabris* and *alleni* groups, from which were calculated the 8 shell variables (Table 5) used for discriminant analysis (Table 7).

Species or subspecies	Pop. no.	Shell no.	Diameter (mm)	Height (mm)	Whorl no.	Striae count*	Basal lip width**			Body whorl depth**		Color rank	Sheen rank	Notes
							At columella	At mid-node	At periphery	Pre-deflection	Behind lip			
alleni alleni	1	1	25.6	15.5	5.2	21	10	12	12	18	27	3	5	subadult
alleni alleni	2	1	27.1	16.6	5.2	18	13	13	14	23	30	3	5	subadult
alleni alleni	3	1	29.3	18.8	5.4	17	25	19	21	23	40	2	5	
alleni alleni	3	2	29.3	19.5	5.4	16	24	17	18	30	41	3	4	
alleni alleni	3	8***	30.0	18.5	5.4	19	21	16	17	23	37	2	5	subadult
alleni alleni	5	A	29.7	18.2	5.4	21	23	14	18	24	32	2	5	
alleni alleni	5	B	31.2	19.5	5.4	16	26	18	20	26	43	3	5	
alleni alleni	6	—	24.0	15.5	5.2	22	19	13	13	20	27	1	5	
alleni alleni	7	—	30.2	18.6	5.4	16	21	18	19	25	37	2	4	
alleni fuscolabris	8	7	32.9	21.1	5.6	17	32	19	20	23	30	3	4	
alleni fuscolabris	8	11	33.4	19.6	5.5	20	27	20	21	25	36	3	4	
alleni fuscolabris	9	1	34.5	20.6	5.5	20	29	18	18	15	32	3	5	
albolabris albolabris	10	A	26.5	17.3	5.7	20	22	21	21	22	32	3	3	
albolabris albolabris	10	B	27.6	18.0	5.8	20	13	13	15	20	33	2	4	subadult
albolabris albolabris	10	C	25.9	16.2	5.4	19	15	16	19	14	22	4	4	
albolabris albolabris	11	3	26.9	17.1	5.8	20	22	20	20	20	33	4	3	
albolabris albolabris	11	4***	28.0	17.5	5.8	21	17	15	16	20	34	4	4	
albolabris albolabris	12	12	29.2	18.3	5.7	23	20	22	22	20	35	5	3	
albolabris albolabris	12	14	28.2	19.3	5.6	18	23	22	22	27	43	4	3	
albolabris albolabris	12	17***	29.7	19.8	5.8	19	23	21	21	24	40	6	3	
albolabris albolabris	13	A	27.0	17.3	5.8	21	22	22	23	20	35	4	4	
albolabris albolabris	13	B	28.8	18.6	5.7	22	25	23	23	24	39	4	3	
albolabris albolabris	14	A	31.3	19.9	5.8	20	27	23	30	21	34	4	3	
albolabris albolabris	14	B	32.1	19.7	5.7	20	24	24	24	20	33	4	3	
albolabris albolabris	14	C	31.6	20.2	5.8	24	24	23	25	24	38	4	4	
albolabris albolabris	15	B	32.2	20.5	5.8	17	23	23	24	27	44	4	3	
albolabris albolabris	15	C	33.5	20.9	5.8	21	25	25	25	26	40	4	3	

albolabris hubrichti	20	1	26.4	17.0	5.6	26	16	14	15	20	34	4	5	
albolabris hubrichti	20	5	23.3	14.3	5.4	22	17	15	16	16	31	4	5	
albolabris hubrichti	20	8	26.6	17.7	5.6	25	18	15	17	21	34	4	4	
albolabris hubrichti	22	A	26.7	15.4	5.2	19	16	13	14	12	29	3	6	
albolabris hubrichti	22	B	26.8	16.9	5.5	21	15	13	16	17	31	3	6	
albolabris hubrichti	23	A	24.2	15.1	5.5	18	12	10	12	14	27	3	6	
albolabris hubrichti	23	B	23.3	15.7	5.4	22	15	13	13	18	30	3	5	
albolabris hubrichti	24	—	23.2	14.0	5.4	21	17	15	13	14	21	2	5	
albolabris hubrichti	25	—	24.3	15.4	5.4	19	10	10	10	16	24	3	6	
major	28	1	33.6	23.0	6.1	21	16	17	19	27	45	4	3	subadult
major	28	23	35.9	24.5	6.0	22	18	23	25	23	42	4	3	subadult
major	28	35	34.4	25.0	6.0	22	34	23	23	26	36	5	2	
major	29	1	32.7	21.9	6.0	23	29	19	21	26	41	4	2	
major	29	2	31.8	21.1	5.9	20	27	19	20	26	40	4	2	
major	29	3	32.6	21.6	5.8	19	30	21	23	24	42	4	1	
major	30	4	31.1	21.0	5.8	22	25	18	19	24	41	4	2	
major	30	6	29.1	18.6	5.3	22	24	19	19	18	33	4	2	
major	30	11	32.2	22.8	5.8	20	27	21	22	28	43	5	2	
major	31	A	29.2	19.5	5.5	21	27	18	17	19	35	5	2	
major	31	B	30.8	21.2	5.7	23	22	16	18	25	34	6	2	subadult
major	31	C	27.9	18.7	5.5	21	20	16	16	22	35	5	2	
solemi	32	—	34.1	23.5	5.7	22	28	23	23	32	51	4	2	
solemi	33	—	24.2	16.2	5.2	21	15	14	14	16	22	6	4	
solemi	34	—	26.0	17.5	5.5	21	13	13	14	23	35	7	4	subadult
solemi	35	—	34.6	24.3	6.1	17	22	18	18	23	44	4	3	
solemi	36	A	25.0	15.5	5.1	16	18	15	14	11	25	5	4	
solemi	36	B	23.7	16.3	5.6	21	20	20	17	20	32	4	3	
solemi	36	C	26.8	18.6	5.6	21	18	16	16	21	30	4	4	

**Number of striae per 2.6 mm on the upper surface of the end of the fifth whorl.*
***Conversion factor = .0325 mm per unit.*
****Shell misclassified by the discriminant function.*

TABLE 7. Linearized discriminant function for the *Neohelix albolabris* and *alleni* groups, based on 8 shell variables (Table 5) standardized to mean = 0 and standard deviation = 1.

	Neohelix species and subspecies					
	alleni		*albolabris*			
Shell variable	*alleni*	*fuscolabris*	*albolabris*	*hubrichti*	*major*	*solemi*
STRIAE	−1.2	−.4	−.2	.8	.7	−.7
BROWN	−3.5	−1.5	1.1	−.9	.8	1.9
GLOSSY	5.1	1.9	−.5	8.0	−10.1	−.1
RELSPIRE	.5	−1.7	−.8	.6	−.0	1.5
WHRLEXPN	1.5	4.2	−.4	−1.9	.8	−1.5
RELNODE	3.1	5.7	−2.8	−1.8	3.0	−1.9
RELLIP	1.1	−.0	2.3	−.6	−2.5	−1.5
RELDEFL	−.6	−1.9	−.3	1.2	.2	.1
Constant	−9.1	−14.4	−4.1	−9.0	−10.4	−5.2

A prediction of this hypothesis is that reproductive character displacement at the level of populations within a species should be no more than moderate, and more likely should be slight to negligible. A test of this prediction is afforded by the results of Table 10. For 12 species, penial morphology was compared between populations sympatric vs. allopatric with a species of similar shell size and shape. In not one of these comparisons was there any detectable difference. Thus the prediction is strongly confirmed, and the hypothesis is supported that differentiation due to reproductive character displacement is moderate at most.

Major differentiation appears to occur uncommonly and rapidly in isolates, because the 6 pairs of greatly different sister taxa are all parapatric, and because the other 14 pairs of isolated (non-sympatric) sister taxa are either identical or only slightly different. Thus the evolutionary pattern suggests a punctuated process: when differentiation does occur in isolates, it is extreme and rapid, leaving no intermediates.

If this hypothesis concerning major differentiation in the genitalia of eastern triodopsines is correct, then what evolutionary mechanisms produce this punctuated process? Since it occurs in parapatry, genetic drift in rare peripheral populations may have sidetracked the selectively canalized developmental program which ordinarily blocks change. Once canalization was overcome, evolutionary change could proceed by any of a number of possible mechanisms, including selection for functional optimization, sexual selection, reproductive character displacement, direct environmental selection, continued genetic drift, genetic linkage, and pleiotropism. Of these, selection for functional optimization and sexual selection seem the most likely causes of major genitalic differentiation.

Selection for functional optimization could have acted to prevent the loss of sperm during transfer due either to (1) the penis slipping out or (2) the sperm being captured and digested by the mate's gametolytic gland (the spermatheca—see Tompa, 1984). These two selective pressures would have favored both sculptural modifications which improved the penis's frictional hold within the mate's gametolytic duct (the functional vagina), and structural modifications which improved the ejected sperm's chances of escaping back down this duct to reach the fertilization pouch (the talon—see Tompa, 1984). It seems reasonable that these selective pressures were responsible for such conspicuous features as (1) the grappling-hook-like pilaster of the *Triodopsis tennesseensis* group (Fig. 11b–d); (2) the chevron-like patterns of wall pustules convergent among the *Xolotrema fosteri-denotata* (Figs. 7, 8), the *Triodopsis platysayoides* (Fig. 12), and the *Triodopsis cragini-tridentata-juxtidens* (Figs. 13–18) lineages; (3) the backward-directed verge in *Webbhelix* (Fig. 6a) and *Neohelix* (Figs. 2–5); and (4) the clubbed apex with a subterminal ejaculatory pore convergent among the *Neohelix solemi* (Fig 6b), the *Xolotrema denotata* (Fig. 7), the *Triodopsis vulgata* (Fig. 9), and the *Triodopsis tridentata-fallax-juxtidens* (Figs. 14–18) lineages (see Fig. 50). Convergences in these structures probably indicate that they are adaptive.

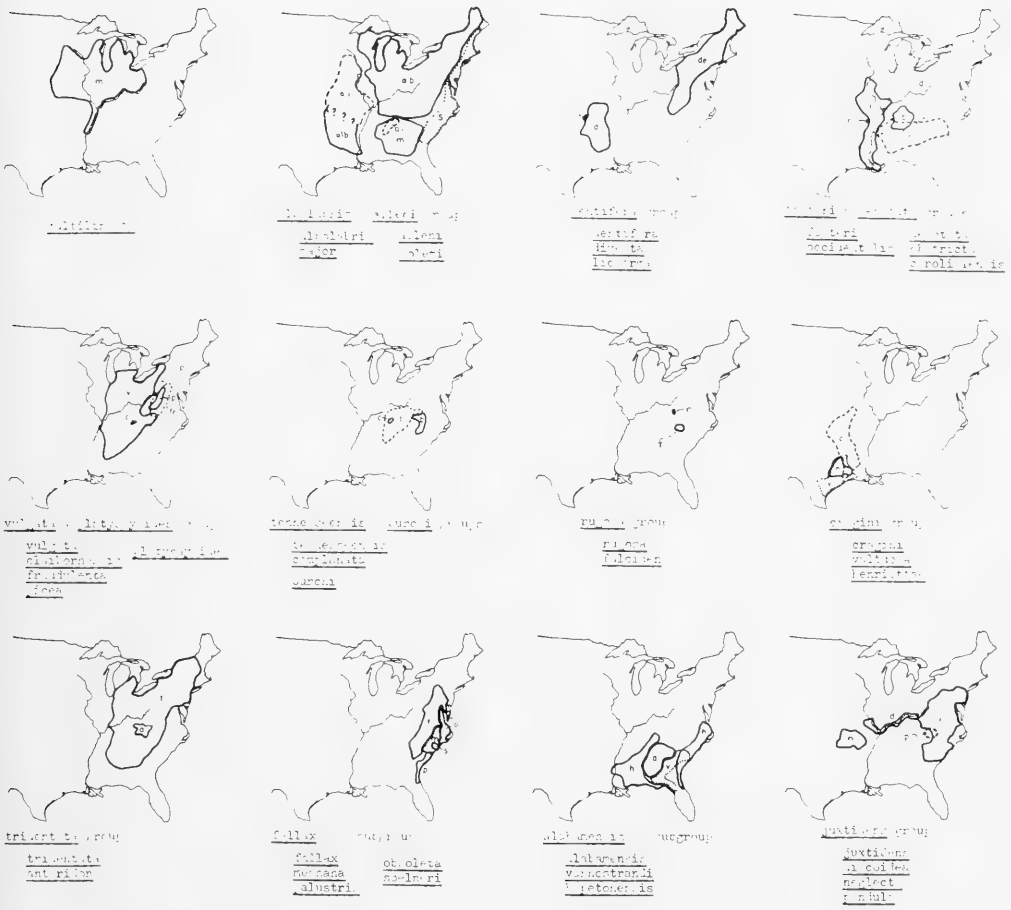

FIG. 49. Range maps of eastern American triodopsines. Adapted from Hubricht (1985).

If these suggestions are correct, why, then, is there so much diversity of form? That is, why are so many different responses to the same two selective pressures? In the first place, the selective pressures may not be equal in each clade. For example, one clade may have a thick mucus which would clog delicate sculpture and therefore would select for coarse sculpture. Or, for example, clades may differ in the strength of the digestive enzymes secreted by the gametolytic gland or in the presence or strength of a muscular pump in the wall of the gametolytic duct (the functional vagina), therefore selecting differently for morphological "strategies" to channel the ejaculate back down the duct.

According to the hypothesis of sexual selection by female choice, "male genitalia function as 'internal courtship' devices used by females to discriminate among "males" (Eberhard, 1986). Runaway sexual selection produces rapid and *arbitrary* divergence in penial morphology, according to Eberhard's model. Much of the divergence in eastern-triodopsine genitalia, however, is not arbitrary but is convergent, suggesting natural selection for function rather than sexual selection. Both forms of selection have probably played a role, however. Certainly the apparent rapidity of divergence is consistent with Eberhard's model.

Reproductive character displacement has already been ruled out as a likely cause of major differentiation.

TABLE 8. Comparison of the revised classification of eastern American triodopsines with three previous classifications.

This classification				Pilsbry's (1940) classification			Webb's (1952, 1954a, 1959) classification			Vagvolgyi's (1968) classification		
Genus	Species group	Species subgroup	Species	Subgenus	Species	Subspecies	Genus	Subgenus	Section	Subgenus	Species	Subspecies
Webbhelix	—	—	*multilineata*	*Neohelix*	*multilineata*	—	*Xolotrema*	*Neohelix*		*Neohelix*	*multilineata*	
Neohelix	*albolabris*	—	*albolabris*	*Neohelix*	*albolabris*	*albolabris*	*Xolotrema*	*Neohelix*		*Neohelix*	*albolabris*	*albolabris*
Neohelix	*albolabris*	—	*major*	*Neohelix*	*albolabris*	*major*	*Xolotrema*	*Neohelix*		*Neohelix*	*albolabris*	*major*
Neohelix	*alleni*		*alleni*	*Neohelix*	*albolabris*	*alleni & fuscolabris*	*Xolotrema*	*Neohelix*		*Neohelix*	*albolabris*	*alleni*
Neohelix	*alleni*	—	*solemi*	*Neohelix*	*albolabris*	*traversensis*		—	—	—		
Neohelix	*dentifera*	*dentifera*	*dentifera*	*Neohelix*	*dentifera*	—		—	—	*Neohelix*	*dentifera*	
Neohelix	*dentifera*	*divesta*	*divesta*	*Neohelix*	*divesta*	—	*Xolotrema*	*Neohelix*	—	*Neohelix*	*divesta*	
Neohelix	*dentifera*	*divesta*	*lioderma*	*M. (Mesodon)*	*indianorum*	*lioderma*	—	—	—			
Xolotrema	*fosteri*	—	*fosteri*	*Xolotrema*	*fosteri*	—	*Xolotrema*	*Wilcoxorbis*	—	*Xolotrema*	*fosteri*	*fosteri*
Xolotrema	*fosteri*	—	*occidentalis*	*Xolotrema*	*obstricta*	*occidentalis*	—	—	—	*M. (Patera)*	*sargentianus*	*occidentalis*
Xolotrema	*denotata*	—	*denotata*	*Xolotrema*	*notata*	—	*Xolotrema*	*Xolotrema*	—	*Xolotrema*	*obstricta*	*denotata*
Xolotrema	*denotata*	—	*obstricta*	*Xolotrema*	*obstricta*	—	—	—	—	*Xolotrema*	*obstricta*	*obstricta*
Xolotrema	*denotata*	—	*caroliniensis*	*Xolotrema*	*caroliniensis*	—	—	—	—	*Xolotrema*	*obstricta*	(hybrid)
Triodopsis	*vulgata*	*vulgata*	*vulgata*	*Triodopsis*	*fraudulenta*	*vulgata*	*Triodopsis*	*Triodopsis*	*Shelfordorbis*	*Triodopsis*	*neglecta*	*vulgata*
Triodopsis	*vulgata*	*vulgata*	*claibornensis*	—	—	—	—	—	—	*Triodopsis*	*neglecta*	*vulgata*
Triodopsis	*vulgata*	*fraudulenta*	*fraudulenta*	*Triodopsis*	*fraudulenta*	—	—		—	*Triodopsis*	*fraudulenta*	—
Triodopsis	*vulgata*	*fraudulenta*	*picea*	—	—	—	—			*Triodopsis*	*fraudulenta*	—
Triodopsis	*platysayoides*		*platysayoides*	*Triodopsis*	*platysayoides*	—	—	—	—	*Triodopsis*	*complanata*	*platysayoides*
Triodopsis	*burchi*	—	*burchi*	—	—	—	—	—	—	*Triodopsis*	*burchi*	
Triodopsis	*tennesseensis*	—	*tennesseensis*	*Triodopsis*	*tridentata*	*tennesseensis*	—	—	—	*Triodopsis*	*complanata*	—
Triodopsis	*tennesseensis*	—	*complanata*	*Triodopsis*	*tridentata*	*complanata*	—	—	—	*Triodopsis*	*complanata*	—
Triodopsis	*rugosa*	—	*rugosa*	*Triodopsis*	*rugosa*	—	—	—	—	*Triodopsis*	*rugosa*	—
Triodopsis	*rugosa(?)*	—	*fulciden*	—	—	—	—	—	—	*Triodopsis*	*fulciden*	—
Triodopsis	*cragini*	—	*cragini*	*Triodopsis*	*cragini*	—	*Triodopsis*	*Haroldorbis*	—	*Triodopsis*	*copei*	*cragini*
Triodopsis	*cragini*	—	*vultuosa*	*Triodopsis*	*vultuosa*	—	—	—	—	*Triodopsis*	*copei*	(hybrid)
Triodopsis	*cragini*	—	*henriettae*	*Triodopsis*	*vultuosa*	*henriettae*	—	—	—	*Triodopsis*	*copei*	(hybrid)
Triodopsis	*tridentata*	—	*tridentata*	*Triodopsis*	*tridentata*	—	*Triodopsis*	*Triodopsis*	—	*Triodopsis*	*tridentata*	—
Triodopsis	*tridentata*	—	*anteridon*	*Triodopsis*	*rugosa*	*anteridon*	—	—	—	*Triodopsis*	*rugosa*	—
Triodopsis	*fallax*	*fallax*	*fallax*	*Triodopsis*	*fallax*	—	—	—	—	*Triodopsis*	*fallax*	*fallax*
Triodopsis	*fallax*	*fallax*	*messana*	—	—	—	—	—	—	*Triodopsis*	*fallax*	(hybrid)
Triodopsis	*fallax*	*fallax*	*palustris*	—	—	—	—	—	—	*Triodopsis*	*fallax*	*obsoleta*
Triodopsis	*fallax*	*fallax*	*obsoleta*	*Triodopsis*	*hopetonensis*	*obsoleta*	—	—	—	*Triodopsis*	*fallax*	*obsoleta*
Triodopsis	*fallax*	*fallax*	*soelneri*	*Triodopsis*	*soelneri*	—	—	—	—	*Triodopsis*	*soelneri*	—
Triodopsis	*fallax*	*alabamensis*	*alabamensis*	*Triodopsis*	*vannostrandi*	*alabamensis*	—	—	—	*Triodopsis*	*fallax*	*alabamensis*
Triodopsis	*fallax*	*alabamensis*	*vannostrandi*	*Triodopsis*	*vannostrandi*	—	*Triodopsis*	*Triodopsis*	—	*Triodopsis*	*fallax*	(hybrid)
Triodopsis	*fallax*	*alabamensis*	*hopetonensis*	*Triodopsis*	*hopetonensis*	—	*Triodopsis*	*Triodopsis*	—	*Triodopsis*	*fallax*	(hybrid)
Triodopsis	*juxtidens*	*juxtidens*	*juxtidens*	*Triodopsis*	*tridentata*	*juxtidens*	*Triodopsis*	*Triodopsis*	—	*Triodopsis*	*juxtidens*	*juxtidens*
Triodopsis	*juxtidens*	*juxtidens*	*discoidea*	*Triodopsis*	*tridentata*	*discoidea*	*Triodopsis*	*Triodopsis*	—	*Triodopsis*	*juxtidens*	*discoidea*
Triodopsis	*juxtidens*	*neglecta*	*neglecta*	*Triodopsis*	*neglecta*	—	*Triodopsis*	*Triodopsis*	—	*Triodopsis*	*neglecta*	*neglecta*
Triodopsis	*juxtidens*	*neglecta*	*pendula*	—	—	—	—	—	—	*Triodopsis*	*pendula*	—

TABLE 9. Comparison of the difference in penial morphology with the relationship between geographic ranges for 25 pairs of sister taxa of eastern triodopsines according to the phylogeny in Fig. 50. The taxa are designated by five-letter abbreviations. Question marks denote pairs of phylogenetically uncertain status. "−" is a minus sign.

Phylogenetically adjacent taxa	Penial shift	Geographical relationship
solem vs. rest of Neohelix	great	parapatric
fostr group vs. denot group	great	parapatric
platy vs. vulgt group	great	parapatric
tenns group vs. burch group (?)	great	parapatric
cragn group vs. rugos group	great	parapatric
cragn group vs. tridt group	great	parapatric
Webbhelix vs. Neohelix-solem	moderate	sympatric
soeln vs. rest of fallx subgroup	moderate	sympatric
tridt group vs. juxtd group	moderate	sympatric
dentf group vs. albol group + allen	moderate	sympatric
albol group vs. allen	moderate	sympatric
albol vs. major	slight	allo or parapatric
dentf group vs. divst group	slight	allopatric
vulgt subgroup vs. fraud subgroup	slight	allo or parapatric
rugos vs. fulcd (?)	slight	allopatric
liodm vs. divst	none	peripatric
occdt vs. fostr	none	peripatric
denot group (3 spp.)	none	paraipatric
claib vs. vulgt	none	peripatric
picea vs. fraud	none	peripatric
compl vs. tenns	none	peripatric
cragn group (3 spp.)	none	peripatric
fallx subgroup-soeln (7 spp.)	none	parapatric
anter vs. tridt	none	peripatric
juxtd group (4 spp.)	none	peri or allopatric

Did the external environment select for penial-morphological differences in the eastern triodopsines? It seems unlikely. The species groups and genera—that is, the major morphological types—do not segregate ecologically (Emberton, 1986), nor is there any evidence of environmental correlation at any level, including within species groups. There seems to be no correlation between the size of the penis and its structural complexity. For example, *Neohelix lioderma* is as small in both body and penis as many species of *Triodopsis,* yet has the *Neohelix* penial sculpture in its full complexity (Fig. 5a). A correlation recurrent in stylommatophorans between arid habitat and short penial length (Solem, personal communication) does not apply to the eastern triodopsines, in which the greatest penis-length-to-shell-diameter ratio occurs in the *Triodopsis cragini* group (Fig. 13), which also occupies the most arid habitat of all known triodopsines (Emberton, 1986).

Genetic drift, although possibly the instigator of genitalic divergence by straying from canalized fitness peaks, is not likely to be the proximate cause of the divergence. Evidence for this view lies in the multiple convergences and in the apparent speed and morphological precision of evolution. Drift, however, can be held responsible for vestigialization: random variation in structures that are no longer functional. The dorsal pilaster of *Neohelix solemi* (Fig. 6b), as well as the verges of *N. solemi,* the *Xolotrema fosteri* group (Fig. 8), and the *Xolotrema denotata* group (Fig. 7), are presumably vestigial.

Although the rough concordance between conchology and penial morphology (Fig. 50) could indicate genetic linkage, with selective changes in the shell randomly inducing unselected changes in the penis, it is more likely that since both shell and genitalia have undergone (independent) evolutionary divergence, they both are correlated with time, and hence, secondarily, with each other. Eastern triodopsines have a relatively high chromosome number (29 to 32 pairs, according to Husted & Burch, 1947), obviating the ne-

TABLE 10. The localities (state:county) of populations dissected in searches for reproductive character displacement between pairs of conchologically similar species of eastern American triodopsines. The number of specimens dissected from each population is in parentheses.

Species A	Allopatry	Sympatry	Allopatry	Species B
albolabris	AR:Logan(1) AR:Washington(1) OK:Sequoyah(3) TX:Houston(3) LA:Washington(1)	AR:Crawford(2,3)	AR:Izard(8) IA:Lynn(1) IA:Jackson(1) IA:Clayton(1)	*alleni*
albolabris	WV:Greenbrier(2) WV:Boone(3) NC:Watauga(3) OH:Athens(2) PA:Chester(2)	WV:Preston(3,3)	WV:Pendleton(3)	*dentifera*
vulgata	KY:Harlan(4) TN:Morgan(2)	KY:Fayette(1,3&3)	—	*denotata* & *tennesseensis*
vulgata	KY:Fayette(1) TN:Morgan(2)	KY:Harlan(1,4)	KY:Edmonson(2) WV:Pendleton(1) WV:Pocahontas(1) WV:Preston(1) OH:Athens(3)	*tridentata*
tridentata	KY:Harlan(1) WV:Pocahontas(1) WV:Pendleton(1) WV:Preston(1) OH:Athens(3)	KY:Edmonson(2,2)	—	*obstricta*
tridentata	WV:Pocahontas(1) WV:Preston(1) KY:Edmonson(2) KY:Harlan(1) OH:Athens(3)	WV:Pendleton(1,2)	—	*picea*
juxtidens	WV:Pendleton(2) NC:Catawba(3) NC:Burke(1)	WV:Pocahontas(2,1)	WV:Pendleton(1) WV:Preston(1) KY:Edmonson(2) KY:Harlan(1) OH:Athens(3)	*tridentata*

cessity for, or the probability of, tight linkages.

Pleiotropy is also an unlikely explanation for the major morphological diversity of the eastern-triodopsine penis because the penis develops from mesoderm, whereas the shell-forming mantle develops from ectoderm (Raven, 1975).

To summarize, neither reproductive character displacement, environmental selection, genetic drift, genetic linkage, nor pleiotropy is a probable cause of major evolutionary change in eastern-triodopsine genitalia. This supports the suggestion that selection for functional optimization and sexual selection are the most likely causes.

Shell evolution: pattern and process

Since the consensus phylogeny (Figs. 28, 50) was constructed strictly from soft-part anatomy and biochemistry, there is no circularity in using it to detect patterns of shell evolution. Shell variation was analyzed at three taxonomic levels: among genera, among species groups, and within species groups. Patterns—and inferred processes—of variation differ among these levels.

In general, each genus has a characteristic shell morphology (Fig. 50). *Neohelix* and *Webbhelix* share the plesiomorphous shell: large, globose, and toothless. *Xolotrema* shells are medium-sized and depressed, with

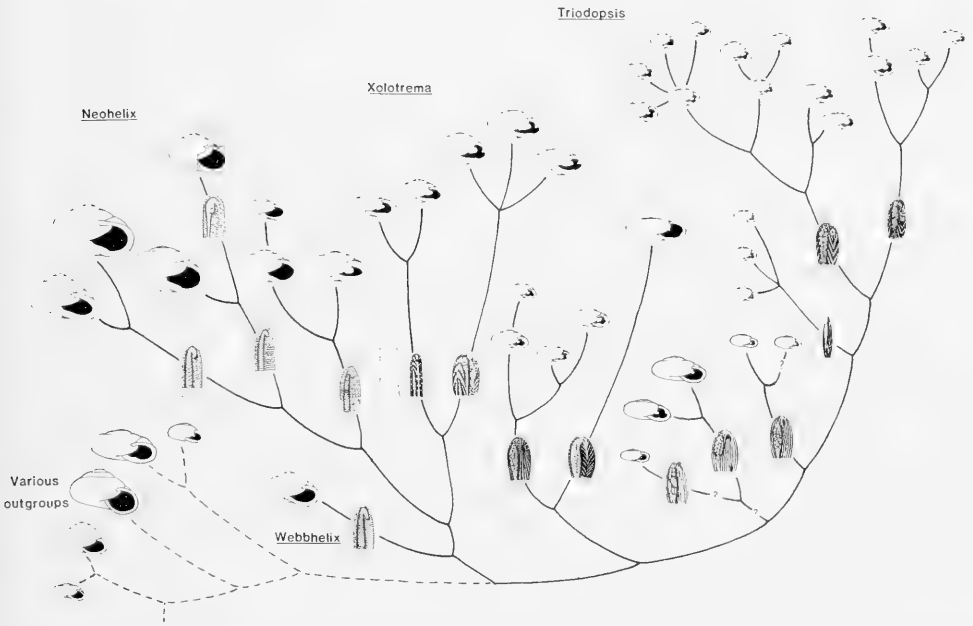

FIG. 50. Evolution of shell morphology and of upper penial sculpture in eastern American triodopsines.

a blade-like parietal tooth and a long basal lamella. *Triodopsis* shells are small, subglobose, and tridentate. The most common exceptions to this generality are in size. Intraspecific variation—discussed below—produces broad overlap in shell size, both within and between genera. Nevertheless, the largest occur in *Neohelix,* and by far the smallest occur in *Triodopsis.* The rare convergences (*Neohelix dentifera, Triodopsis platysayoides,* and the *T. tennesseensis* group) offer little contradiction to the generality that within major clades (genera) of eastern triodopsines, shell morphology is distinct and virtually static.

The evolutionary process behind this pattern is problematic. A preliminary study (Emberton, 1986) concluded that the genera broadly overlap ecologically, with virtually no conchological changes accompanying ecological convergences. Ecological relationships are clearly in need of further investigation (see Goodfriend, 1986).

Within a genus, the distributional pattern of shell characters among species groups is generally mosaic, with many cases of convergence or parallelism. This rank mosaicism has confused past conchologically based systematics (e.g. Pilsbry, 1940, and Vagvolgyi, 1968; see Table 8). Its pattern suggests a process encompassing both drift and selection. Possible selective explanations for a few of the recurrent shell features are discussed in the next section. For many of these features, however, it seems more likely—but would be impossible to demonstrate unequivocally—that their genetic program is ubiquitous in the subgenus, is selectively neutral with respect to its alternative states, and is expressed randomly among species of the clade due to genetic drift. This process is called genetic indeterminism by Throckmorton (1965)—also see Gould's (e.g. Gould & Woodruff, 1986) discussions of morphological canalization.

Within species, there is great variation in shell size, spire height, umbilical relative width, and whorl count (Table 11). Diameter ranges up to 95% within a species; the extent of this range in shell size depends on the number of populations and specimens measured. Such variation in adult size is common not only in land snails, whose time for shell growth (before it is interrupted by reproductive maturity) depends heavily on the local humidity regime (e.g., Solem & Christensen,

TABLE 11. Ranges of variation in shell measurements in species of eastern triodopsines. Compiled from the text of Vagvolgyi (1968), with taxonomic adjustments according to Table 8.

Species	Lots	Shells per lot	Total shells	Diameter	Diameter range	Height	Ht/diam	Umbilicus	Um/diam	Whorls	Whls/diam
multilineata	32	2–25	230	16.2–28.1	73.5%	10.0–18.2	.55–.71	0	0	—	—
albolabris + (solemi)	86	1–21	468	20.2–35.6	76.2%	11.5–23.9	.52–.73	0	0	—	—
major + (solemi)	19	1– 8	41	27.1–41.7	53.9%	18.8–30.5	.62–.74	0	0	—	—
alleni	10	2–17	57	23.3–30.1	29.2%	13.7–17.8	.55–.64	0	0	—	—
dentifera	13	1– 5	24	19.5–27.6	41.5%	10.6–15.6	.52–.57	0	0	—	—
divesta	7	1–13	24	16.9–19.5	15.4%	8.7–11.8	.49–.67	0	0	—	—
fosteri	23	2–12	116	13.8–22.0	59.4%	6.9–11.0	.43–.58	0	0	4.6–5.5	.25–.35
denotata	42	2–24	197	17.0–25.9	52.4%	8.5–12.9	.42–.57	0	0	4.8–5.8	.21–.30
obstricta	11	2– 7	41	19.7–25.9	31.5%	9.6–12.6	.41–.56	0	0	5.2–5.8	.22–.27
vulgata + claibornensis	37	1–25	231	12.2–19.8	62.3%	5.8–10.6	.41–.63	2.4–5.1	.16–.30	5.0–6.4	.30–.45
fraudulenta + picea	9	1–10	34	12.7–16.9	33.1%	6.7– 9.0	.45–.63	1.9–3.9	.15–.24	5.2–6.1	.34–.43
burchi	3	2–15	29	8.9–13.2	48.3%	4.9– 6.2	.45–.56	1.5–2.7	.15–.21	—	—
tennesseensis + complanata	22	1–11	65	16.7–23.3	39.5%	8.0–10.6	.40–.54	2.9–6.0	.17–.26	—	—
rugosa + anteridon	13	1– 8	44	10.6–15.5	46.2%	5.4– 7.2	.42–.54	1.7–3.6	.16–.23	—	—
cragini	16	2–12	83	7.7–10.8	40.3%	3.7– 6.7	.48–.67	1.1–2.0	.13–.19	4.3–5.5	.47–.60
vultuosa	5	2– 7	16	11.5–14.5	26.1%	6.1– 7.5	.48–.60	1.9–4.0	.17–.28	5.3–6.0	.41–.50
tridentata	80	1–15	395	12.3–20.7	68.3%	5.5–11.0	.43–.57	2.0–4.3	.14–.24	—	—
fallax	20	2–30	187	10.1–15.5	53.5%	5.9– 9.1	.50–.66	1.3–3.2	.12–.22	5.0–6.6	.38–.56
palustris + obsoleta + (fallax)	24	2–31	434	9.7–13.5	39.2%	5.1– 8.8	.47–.65	1.2–2.7	.11–.22	4.5–6.0	.39–.53
alabamensis	15	2–27	144	8.6–13.1	52.3%	4.9– 7.5	.48–.66	1.2–3.0	.12–.24	5.1–7.0	.49–.68
juxtidens	38	1–32	282	9.8–19.1	94.9%	5.3– 9.9	.46–.61	1.1–3.5	.12–.49	—	—
discoidea	13	3–15	94	12.9–20.9	62.0%	5.8–10.0	.40–.54	2.2–4.6	.14–.23	—	—
neglecta	9	1–30	81	9.9–13.2	33.3%	4.6– 6.5	.41–.53	2.1–3.9	.20–.30	4.8–6.0	.40–.51
pendula	9	1–10	34	10.7–13.8	29.0%	5.5– 7.2	.50–.59	2.0–3.2	.19–.25	4.9–5.8	.41–.49

1984; Gould, 1985), but also in aquatic gastropods (e.g., Vermeij, 1980).

Vagvolgyi (1968) documented that intraspecific shell variation is geographically patchy and non-clinal (the small number of clines he reported is no more than one would expect by chance). The same was true of apertural features, keel, fulcrum, and sculpture. Vagvolgyi attributed this patchy variation to ecophenotypic responses to patchily distributed microclimates, "occur[ing] in spite of gene flow, not because of lack of it." This interpretation is probably correct—see the documentation of this phenomenon in *Cerion* (Gould, 1985) and in *Neohelix major* and *Mesodon normalis* (Emberton, 1986))—but genetic drift and local selection could also play significant roles.

In addition to this patchy, non-clinal pattern in size and shape, there are several correlations between niche and shell morphology which recur within species and species groups. These convergences, discussed in turn below, are probably due to environmental selection.

Apertural obstruction correlates with ground moisture. Parallel altitudinal clines in the size of apertural teeth occur in *Triodopsis tridentata, T. fallax,* and *T. fraudulenta* (Vagvolgyi, 1968). The aperture becomes more obstructed with increasing elevation and, concomitantly, increasing ground moisture. An altitudinally opposite cline exists in the *cragini* group (Vagvolgyi, 1968), with the most highly obstructed species *(henriettae)* inhabiting lowland, riverine forests; the least obstructed species *(cragini)* occupying dry uplands; and *vultuosa* intermediate in both apertural obstruction and habitat. Thus the consistent correlation in all four clines is with ground moisture. This pattern supports the view of apertual teeth as barriers to insect predators, presuming that the density and/or diversity of insect predators increases with increasing ground moisture, but fails to support the view of apertural denticles as barriers to water loss (Goodfriend, 1986). An alternative view is that snails living in humid habitats have a longer season of activity, hence more time for the deposition of shell material, including the apertural teeth.

Flatness correlates with crevice dwelling. Five separate species groups show the parallel evolution of a flat-spired species associated with rock crevices (see Emberton, 1986). In the *Neohelix alleni* group, *N. alleni fusco-labris* is flat for the group, and is restricted to limestone-cliff areas of northern Alabama and adjacent Tennessee (Hubricht, 1985, and personal communication; personal observations). The *Xolotrema fosteri* group has *X. occidentalis,* a flat, subcarinate cliff-dweller; the *Xolotrema denotata* group has *X. obstricta,* which, with its pronounced keel and depressed spire, is the most rock-associated member of its group. The aberrant *Triodopsis platysayoides* inhabits crevices between sandstone blocks in a restricted region of the New River Gorge, West Virginia, and has the flattest spire of the entire genus. The *Triodopsis juxtidens* group's only exclusive cliff-dweller (along the Ohio River Valley) is the conspicuously flat *T. discoidea.* This parallel concordance with habitat suggests that a flat shell is adaptive for rock-crevice dwelling. Similar environmental correlations occur in several lineages of Mediterranean helicids, suggesting the same selective pressures (Goodfriend, 1986).

Glossiness correlates with water. Another iterated shell-habitat correlation—pointed out by Vagvolgyi (1968), and comparable, as he stated, to Rensch's (1932) trends—is between a glossy periostracum and nearness to a large body of water. The glossiest member of the *Neohelix dentifera* group *(N. lioderma)* appears to be restricted to the Arkansas River Valley. In the *Triodopsis tennesseensis* group, glossy *T. complanata* lives along the river, whereas dull *T. tennesseensis* occurs on the upper banks and farther inland. In the *Triodopsis fallax* group, two species have independently evolved a shiny periostracum: *T. palustris* along the Santee River floodplain, and *T. soelneri* in the marshes of the Lake Waccamaw area. The riverine cliff snail *Triodopsis discoidea* is the glossiest member of the *T. juxtidens* group. Glossiness may be an exclusively ecophenotypic effect, but is probably at least a partially selected trait. Its heritability has never been assessed, although Grimm's (1975) lab-reared *T. soelneri* and its hybrids should yield important data (see Appendix D).

Juvenile apertural size correlates with aridity. Vagvolgyi (1968) also noted that arid-adapted species of eastern triodopsines have relatively smaller juvenile apertures, hence the tightly coiled shells of the *Triodopsis alabamensis* group and, to a lesser extent, the *Triodopsis cragini* group. The same pattern has been found in various other groups of land snails; it implies natural selection for water loss, although not all experimental re-

sults have been consistent with this interpretation (Goodfriend, 1986).

To summarize, there are two major components to the pattern of shell variation within species and species groups of eastern triodopsines, and they appear to differ in the processes which produced them. First, the patchy, non-clinal variation in the size, and many features of form, of the shells is probably due to ecophenotypic effects. And second, the several correlations between environment and shell morphology iterated among separate lineages are probably due primarily to natural selection, with perhaps some ecophenotypic contribution.

What is a species in the eastern American triodopsines?

If the biological species concept were used for the eastern triodopsines, species groups would probably be reduced to species. Species groups have, with two exceptions *(Neohelix solemi* and *Triodopsis soelneri),* virtually identical genitalia, hence are probably capable of interbreeding. Indeed, hybridization has been reported (based on analysis of geographical shell variation) within the *Xolotrema denotata* group (Vagvolgyi, 1968) and the *Triodopsis fallax* group (Hubricht, 1953; Vagvolgyi, 1968; Grimm, 1975). Vagvolgyi (1968) even concluded that certain Hubrichtian (1985) species are not species at all, but hybrid swarms: *X. caroliniensis, T. vultuosa, T. henriettae, T. messana, T. vannostrandi,* and *T. hopetonensis.* The only reported cases of reproductive isolation within species groups occurs among some members of the *Triodopsis fallax* group, which nevertheless still hybridize in the laboratory (Grimm, 1975; see Appendix D). Laboratory hybridization has also been reported within the *Xolotrema denotata* group (Webb, 1980).

In the revision of the *Neohelix albolabris* and *alleni* groups (Appendix B), the biological species concept was applied, using the "yardstick method" of comparing sympatric species to determine the degree of penial difference capable of reproductively isolating species (under the still unproven assumption that penial morphology is the predominant mate-recognition system). Thus, subspecific status was assigned to genetically isolated, conchologically differentiated taxa which had the same or only minutely different penial sculpture. Specific status was provisionally assigned to *N. major* because its penis may be different enough, by yardstick criteria, from the similar *N. albolabris* to prevent interbreeding, and this difference is disjunct, with no sign of clinal intergradation. If these same criteria were applied to a species-level revision of all eastern triodopsines, then species groups would be reduced to species.

This was not done for a number of reasons. First, there are important precedents in land-snail taxonomy for species which hybridize. Gould & Woodruff (1986), for example, opted to assign specific status to two hybridizing "semispecies" of snails *(Cerion glans* and *gubernatorium)* of New Providence Island because of a multitude of evolutionarily significant differences. Murray & Clarke (e.g., 1980) followed a similar taxonomic path with the "incipient species" of *Partula* on Moorea.

Second, there is some evidence that species may be reproductively isolated despite close genitalic and conchological similarities. Recent discoveries in the confamilial genus *Ashmunella* indicate that morphological differences among valid species can be slight. *Ashmunella* has all appearances of being oversplit, with specific status bestowed on a mosaic collection of often subtle shell difference. Karyotypic and breeding studies (Babraksai & Miller, 1984) have shown, however, that at least one such subtle shell distinction marks true biological species: hybrids of *A. proxima* and *A. lenticula* suffer gametic disgenesis producing effective sterility. Thus, in the eastern triodopsines, Grimm's (1975) and Hubricht's (1953, 1985) assertions that *messana* and *hopetonensis,* as well as *obsoleta* and *hopetonensis,* live in sympatry without conchological evidence of hybridizing cannot necessarily be rejected (as Vagvolgyi, 1968, did) simply because of the subtlety of their shell differences, because apparent integrades exist elsewhere, or because—as reported in this paper—their genitalia appear identical.

In view of these considerations, there simply is not enough evidence on which to base a robust species-level revision of eastern triodopsines at the present time. Therefore Hubricht's (1985) species designations have been retained in the supraspecific revision (Appendix C).

Recommendations for future research

The nature and definition of a species need to be researched for eastern triodopsines. Because this is now one of the phylogeneti-

cally best understood groups of pulmonates, such investigations will yield important generalizations concerning pulmonate systematics.

In addition, despite the general congruence between the two data sets (genitalic and allozymic) used for phylogenetic reconstruction, there are several problematic groups for which data were incomplete or in conflict. (1) The taxonomic status of *Webbhelix multilineata chadwicki* needs to be assessed (see Webb, 1952). (2) The zone of potential contact or integradation between *Neohelix albolabris albolabris* and *N. major* needs collection and assessment. (3) The precise ranges of *N. albolabris hubrichti* and *N. alleni alleni,* and the degree of range overlap and sympatry, need to be determined. (4) Topotypic *"Neohelix albolabris traversensis"* needs collection and dissection to test the prediction that it is *N. albolabris albolabris* which conchologically converges on *solemi;* if it is anatomically what has been called *solemi,* then the name *traversensis* has precedence for this species. (5) The status of *Neohelix lioderma* is in question: is it merely a small-sized population of *N. divesta?* (6) The systematic and ecological relationships of *Xolotrema fosteri* and *X. occidentalis* need evaluation; for example, do other *"occidentalis's"* (flat-spired cliff dwellers) occur as ecophenotypic variants within the range of *fosteri?* (7) One of the most promising areas of investigation is in the *Xolotrema denotata* group. Despite a basic sameness of the aperture and of the penial morphology, and despite evidence of hybridization, shell variation is extreme. It ranges from subglobose, with a rounded periphery, bearing periostracal hairs, and ribless *(denotata);* to depressed, with a keeled periphery, hairless, and strongly ribbed *(obstricta).* These are the only hairy shells and the only keeled shells in the eastern American triodopsines. Vagvolgyi's (1968: Fig. 21) claim that *caroliniensis* is a hybrid zone around the circular range of *obstricta* where it is nearly surrounded by the range of *denotata* is quite plausible, but needs to be tested electrophoretically and by more rigorous shell analysis. The ecological significance, if any, of the disjunct shell forms has yet to be investigated. (8) The question of whether *Triodopsis claibornensis* is a local ecophenotypic dwarf of *T. vulgata* needs to be settled. (9) Likewise, what is the status of *Triodopsis picea* in relation to *T. fraudulenta?* Does its ecological separation (high-montane) and its shell differentiation (dwarf, pustulose) denote incipient or full speciation, or ecophenotypic variation? (10) The phylogenetic position of *Triodopsis platysayoides* as sister to the *T. vulgata* group needs corroboration from an independent data set to be considered truly robust, because of its aberrant, unique penial morphology. (11) The electrophoretic similarity of *Triodopsis burchi* to *Neohelix,* in addition to its unique dorsal-pilastral sculpture of uncertain homology, make it a problematic species. It clearly needs further comparisons. (12) The phylogenetic position of the *Triodopsis tennesseensis* group is in question, and needs testing by other data sets. The possibility needs to be investigated that *T. complanata* is an ecophenotypic variant of *T. tennesseensis,* its glossiness due to living near water. It the two are true species, do they hybridize? (13) Electrophoresis of *fulciden* should clarify its now dubious placement in the *rugosa* group. (14) The *Triodopsis cragini* group, despite its disjunctly different penial morphology, parallels the variation of the *T. fallax* subgroup. Vagvolgyi's (1968) claim of hybridization, rejected by Cheatum & Fullington (1971) and Hubricht (1985), deserves electrophoretic testing. Any shell studies should explore ecological correlations. (15) Since *Triodopsis anteridon's* range lies within that of *T. tridentata,* is it an ecological variant (confusingly convergent, by the way, on *T. rugosa*)? If not, do the two species interact? (16) The Grimm-Hubricht hypothesis on the evolution of the *fallax* subgroup (Appendix D) needs rigorous testing. Grimm's unpublished lab-hybridization data and specimens should be evaluated. Multivariate shell morphometrics, coupled with targeted mitochondrial DNA studies, should resolve the problem of this intriguing evolutionary microcosm. (17) The *Triodopsis juxtidens-T. discoidea* pair seems to be a case of incipient or recent speciation involving a major shift in habitat accompanied by an apparently adaptive shell change. Vagvolgyi (1968) claimed conchological intermediates between *juxtidens* and *discoidea* in the Kanawha River Valley of West Virginia, suggesting that speciation is not complete. Careful investigation of this system, including ecological analyses and tests for directional selection for a flattened spire may be the best approach to generalities about the speciation process in triodopsines.

The eastern triodopsines, because of their species diversity, their robust phylogenetic

hypothesis, their mapped species' ranges, and their broad conchological, genitalic, and allozymic variation, are a superlative system for further evolutionary studies. For example, the three major clades *(Neohelix, Triodopsis,* and *Xolotrema)* could be compared as to (1) their modes of speciation; (2) their covariations among the respective evolutionary rates of anatomy, shell, and allozymes; (3) their phylogenetic changes in shell ontogeny, as measured from sections or x-rays of adult shells (Raup, 1966; Schindel, in review, 1986); (4) their rates of spread from Pleistocene refugia as determined by allozymic geographic variation; (5) their strengths of selection—measured by comparing dead shells of juveniles with the juvenile whorls of living adults—in parallel adaptive trends (e.g., flattening of the spire as an adaptation for cliff dwelling); and (6) their ecophenotypic plasticity of shell shape.

Perhaps the most promising aspect of the eastern triodopsines for the study of evolution is that their conchological radiation has been reiterated by the distantly related, confamilial genus *Mesodon* (Pilsbry, 1940; Emberton, 1986). These two radiations overlap each other almost perfectly in geography, ecology, conchology, and species richness (Emberton, 1986). Thus *Mesodon* represents a natural, in situ replication of the evolution of the eastern triodopsines. Such synchronous, sympatric, parallel radiations appear to be quite rare in nature, and present untapped opportunities for formulating and testing general hypotheses concerning evolutionary convergence. Since convergence can only be evaluated in the context of phylogeny (e.g., Bookstein et al., 1986), this monograph and a parallel monograph in progress on *Mesodon* lay groundwork for utilizing this system.

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APPENDIX A. ELECTROPHORETIC PROCEDURES

Loci. The 16 loci are listed in Table 12.

Gels. 33 grams starch (Electrostarch Company, lot #392) to 250 ml buffer. Dimensions 18.4 mm × 14.4 mm × 0.6 mm.

Paper wicks. Cut from filter paper, dimensions 7–8 mm × 1.2–1.3 mm; 25–30, rarely up to 40, per gel.

Buffer systems and running times. All were run at 35 milliamperes or 350 volts, whichever was reached first. TC-6, Tris-Citrate pH6 (Shaw & Prasad, 1970): 2.5 hr. Poulik (discontinuous tris-citrate): 3.5 hr. TEB 9, tris-EDTA-borate pH 9.1 (Ayala *et al.,* 1973): 4.5 hr. TEB 9/8, TEB 9 gel run in TEB 8 (Shaw & Prasad, 1970) tray buffer.

Power supply. Heath Schlumberger Regulated High Voltage Power Supply; each gel run in a separate tray under a separate power supply.

Grinding buffer. Modified from Selander et al. (1971): 0.01 molar Tris buffer, 0.001 molar EDTA, 5×10^{-5} molar NADP, 0.2 parts per thousand beta-mercaptoethanol, pH adjusted to 6.8. For making 500 ml: 0.6055 g Tris, 0.1681 g EDTA, 19.1 ml NADP, 0.01 ml beta-mercaptoethanol. Also used de-ionized distilled water for some tissues, with no detectable difference in results.

Chemicals. All from Sigma Chemical Company.

Staining. Recipes from Shaw and Prasad (1970) unless otherwise indicated in Table 12. Stained in a tray for Got and Lap; for all others, stained using agar overlay: 10 ml of 2% agar solution (4 grams agar to 200 ml water) at 60°C per 10 ml of stain, freshly mixed at room temperature. Agar overlays conserve staining chemicals and allow the gel to be readon a light table as staining proceeds, allowing greater scoring accuracy.

Controls. Mesodon zaletus from the population at Monte Sano, Alabama (GS 20 = GS 101) was used as control on all but two runs which used the same species from White Oak Sink, Tennessee (GS 9). The runs made in 1982 had 5 controls in the center of each gel, and the runs made in 1983 had 2 controls on each end and 3 controls in the center of each gel.

Scoring. Banding patterns on gels were measured on a light table and immediately copied onto graph paper to the nearest 0.5 mm, with compensations for apparent edge effects and local distortions. Questionable bands were labeled as such on the graph paper record to aid later interpretation.

APPENDIX B. SYSTEMATIC REVIEW OF THE *NEOHELIX ALBOLABRIS* AND *ALLENI* GROUPS

The studied populations are numbered from 1 to 46 as they appear in Fig. 47.

Species group *Neohelix albolabris*
(Figs. 2d-g, 4, 29c–d, 30c–d; Tables 2, 4, 6, 7; Fig. 49)

Key characters. Penis: pilastral lappets approximately half the number of columns of wall pustules; pilaster moderately wide; wall pustules all distinct and approximately equal in size; verge large; retractor-muscle's origin distant (ca 1/2 the penial length) from the penial apex; vas deferens more than 4 times as long as the penis.

Neohelix albolabris (Say, 1816)
(Figs. 2d–g, 29c–d; Tables 2, 4, 6, 7; Fig. 49)

TABLE 12. Enzyme systems used for electrophoretic analysis.

Name	Abbreviation	Enzyme commission number	Buffer system used	Molecular structure(s)	Number of readable loci
Sorbitol dehydrogenase[1]	Sordh	1.1.1.14	TEB 9	Tetramer	1
Malate dehydrogenase[2]	Mdh	1.1.1.37	TC 6	Dimers	2
Malic enzyme[3]	Me	1.1.1.40	TEB 9	Tetramer	1
Isocitrate dehydrogenase[4]	Icd	1.1.1.42	TC 6	Dimer	1
Phosphogluconate dehydrogenase[5]	Pgd	1.1.1.44	TEB 9/8	Dimer	1
Glucose-6-phosphate dehydrogenase[6]	Gd	1.1.1.49	TEB 9/8	Dimers	2
Superoxide dismutase[7]	Sod	1.15.1.1	TEB 9/8	S-1 Dimer S-2 Tetramer	2
Glutamate-oxaloacetate transaminase[8]	Got	2.6.1.1	TEB 9	Dimers	2
Phosphoglucomutase[9]	Pgm	2.7.5.1	Poulik	Monomer	1
Leucine aminopeptidase[10]	Lap	3.4.1.1	TC 6	Monomer	1
Mannose phosphate isomerase[11]	Mpi	5.3.1.8	TEB 9	Monomer	1
Glucose phosphate isomerase[12]	Gpi	5.3.1.9	Poulik	Dimer	1
Total					16

[1]Stains slowly, streaks a bit.
[2]Clear, stains in a few minutes, keeps well.
[3]Stain: 5 ml HCl developer, 5 ml MDH substrate solution, $MgCl_2$, MTT, NADP (0.15 ml), PMS, 10 ml agar. Stains slowly, keeps well. A second locus comes up with TC 6, but is unreliable.
[4]Stains very slowly, streaks a bit. Second locus visible but too streaked to read.
[5]Must be read quickly, blurs badly if left too long.
[6]Second locus does not appear unless 5 mg NADP is added to gel before degassing, as per Brewer (1970). First locus blurs and streaks quickly, second is slow and keeps well.
[7]Comes up slowly. Better if left under fluorescent light. Disappears with time.
[8]Sometimes called asparate amino transferase. Stained in tray, recipe from Selander *et al.* (1971). Soluble (anodal) locus stains faster than mitochondria (cathodal) locus. Both streak, but in one direction, so clearly readable.
[9]Strong satellite bands which had to be learned and discounted. A second locus is clear, but with too much overlap with the first locus to be scored.
[10]Stained in tray. Stains very slowly and keeps well. A second, slow locus is too streaked to read reliably.
[11]Stain recipe from Nichols, Chapman & Ruddle (1973). Stains at a moderate rate, keeps well.
[12]Stains quickly and soon blurs with formation of satellite bands.

Comparisons

Penis. On its pilaster *albolabris* differs from *major* by the density of lappets, having more per unit length (Table 4); and by the shape of the pilastral lappets, having slightly as opposed to greatly convex surfaces (Fig. 2d, e vs. Fig. 4). The wall pustules of *albolabris* are smaller than in *major* (Table 4).

Shell. *N. albolabris* has fewer striae per unit distance than *major* (Table 7). It also differs from *major* in having a lower whorl expansion rate and a much smaller baso-columellar lip node (Table 7).

Key characters

Penis: internal length 10–16 mm; pilaster 1/20th to 1/10th as broad as the penis is long, and bearing 8–14 lappets per 2.6 mm; lappet surfaces slightly convex; wall-pustular columns 16–24 per 2.6 mm; verge 1/7th to 1/5th as long as the penis.

Shell: diameter 23–39 mm, depressed-globose, whorls 5 1/2–6; striae moderately raised, 17–26 per 2.6 mm on the 5th whorl; yellow-brown to brown; glossy to dull; whorls slowly expanding for the group; apertural lip narrow to wide for the group; basocolumellar node absent to inconspicuous; pre-apertural deflection of the body whorl moderate to weak.

Neohelix albolabris albolabris (Say, 1816) (Figs. 2d–g, 29c–d; Tables 2, 4, 6, 7; Figs. 47, 49)

Studied material

(10) OH: Athens County (Ohio 35; FMNH 214917): 12 live adults—dissected #A, B; measured shells #A, B, C. (11) PA: Chester County (GS 129; FMNH 214919): 2 live

adults, 4 tissue samples—dissected #3, 4 (measured #3); electrophoresed #1, 2, 3, 4; measured shells #3, 4. (12) WV: Preston County (GS 130; FMNH 214920); 20 live adults, 20 tissue samples—dissected #9, 11, 14 (illustrated #14); electrophoresed 8, 12, 16, 17, 20; measured shells #12, 14, 17 (illustrated #14). (13) WV: Greenbrier County (GS 139; FMNH 214921): 2 live adults—dissected #A, B; measured shells #A, B. (14) WV: Boone County (GS 142; FMNH 214922): 9 live adults—dissected #A, B, C (measured #A); measured shells #A, B, C. (15) NC: Watauga (GS 151, 152; FMNH 214924): 9 live adults—dissected #A, B, C (measured #A); measured shells #A, B, C.

Published dissections

(0) PA? (Binney, 1851, Plate VI, Fig. IV). (16) NY: Albany County (Simpson, 1901, Plate 8, Figs. 2, 3, 4, 6). (17) PA: Bucks County (Pilsbry, 1940, Fig. 488:7). (18) IN: Monroe County (Webb, 1952), Plate 4, Fig. 12:7). (19) IN: Monroe County (Webb, 1954, Plate 10, Fig. 9:16).

Key characters

Shell: striae 18–23 per 2.6 mm on the 5th whorl; color light to dark brown; surface dull; height to diameter ratio .59–.72; whorls 5.2 to 6.0; outer lip width 15–30 mm; pre-apertural body whorl deflection moderate.

Neohelix albolabris bogani Emberton, new subspecies
(Tables 2, 4, 6, 7; Figs. 47, 49)

Synonymy

Xolotrema albolabris alleni ("Wetherby" Sampson) of Webb, 1952, Gastropodia, 1 (1): 7–8, Figs. 2, 13.

Triodopsis albolabris alleni (Wetherby) of Solem, 1976, Nautilus, 90: 25–36, Figs. 1a, b, 2a, c, 8–12.

Studied material

(23) OK: Sequoyah County (FMNH 176127): 2 live adults—dissected #A, B; measured shells #A, B. (20) TX: Houston County (GS 76; FMNH 214925): 10 live adults, 10 tissue samples—dissected #1, 5, 8. (21) AR: Crawford County (GS 90; FMNH 214926): 2 live adults, 2 tissue samples—dissected #3, 4; electrophoresed #1, 2. (22) AR: Logan County (FMNH 176087): 2 live adults—dissected #A (measured #A); measured shells #A, B. (23) OK: Sequoyah County (FMNH 176144): 1 live adult—dissected; measured shell. (24) AR: Washington County (FMNH 176160): 1 live adult—dissected; measured shell. (25) AR: Washington County (FMNH 176160): 1 live adult—dissected; measured shell. (26) LA: Washington County (FMNH 195989): 1 live adult—dissected; measured shell.

Published dissections

(27) AR: Logan County (Webb, 1952, Plate 4, Fig. 13). (23) OK: Sequoyah County (Solem, 1976, Fig. 5a)—also included in studied material above.

Comparisons

Neohelix albolabris bogani has previously been confused with *Neohelix alleni alleni,* from which it differs by its penial morphology (Figs. 2d vs. Fig. 3; Table 4) and subtle aspects of shell morphology (Table 7). The two occur sympatrically at Devils Den State Park, Crawford County, AR (Fig. 46, populations 3 and 21).

This is the western subspecies of *Neohelix albolabris,* occurring west of the Mississippi from at least Texas to Arkansas, but also getting east of the River in the Delta area (Fig. 46, population 26). It differs from the eastern *N. albolabris* by several shell characters which are convergent on western *alleni:* yellower color, glossier surface, moderately higher spire, and narrower lip (Table 7). It also differs from *N. albolabris* by its denser striae, its slower whorl expansion rate, and its stronger pre-apertural deflection (Table 7). In penial morphology (Table 4) and electromorphs (Table 2, Fig. 27) it shows no significant differentiation from *albolabris albolabris.*

By shell characters, *albolabris bogani* can usually be distinguished from the sometimes sympatric *alleni alleni* by its denser striae, slower whorl expansion rate, smallness of the baso-columellar node, narrower lip, and more pronounced preapertural deflection (Table 7). At Devils Den State Park, *albolabris bogani* was smaller in diameter than *alleni;* it is not known whether they were microsympatric, as the collection covered a wide area of hardwood forest.

Key characters

Shell: striae 18–26 per 2.6 mm on the 5th whorl; color yellow-brown to light brown; surface glossy; height-to-diameter ratio .58–.68; whorls 5.2–5.7.

Remarks

Despite the virtually identical penial morphology and electromorphs, the disjunct shell morphologies and geographic ranges clearly indicate subspecific status for *bogani.* The precise range relationships still need to be worked out (to fill in the gaps in Fig. 47) before sound hypotheses can be formulated about the relative time of separation of the two subspecies of *albolabris,* but it appears likely that the Mississippi River has kept them isolated for the past 20,000–40,000 years (Delcourt & Delcourt 1981). Pilsbry (1940: 842) reported an introduction of *Neohelix albolabris* from North Carolina to Tyler, Texas, presumably in the 1890's (Fig. 47). Although it is tempting to speculate that this was the founder of *albolabris bogani,* both the degree of conchological differentiation and the widespread occurrence of this subspecies in an arid terrain argue strongly against such a theory.

The shell convergence on *alleni alleni* by which *albolabris bogani* has until now escaped detection, is intriguing. Working out the degree of range overlap and sympatry of these two trans-Mississippian species would be a worthy contribution to malacology by providing valuable data on the sympatric-convergent evolution of shell morphology and color.

This subspecies is named for Dr. Arthur Bogan of the Department of Malacology, Academy of Natural Sciences of Philadelphia.

Neohelix major (Binney, 1837)
(Figs. 4, 30c, d; Tables 2, 4, 6, 7; Figs. 47, 49)

Studied material

(28) TN: Blount County (GS-3; FMNH 214927): 7 live adults, 7 tissue samples—dissected #35; electrophoresed #1, 6, 7, 10, 22, 24, 28; measured shells #1, 3, 35. (29) TN: Meigs County (GS-105; FMNH 214928): 3 live adults, 3 tissue samples—dissected #2, 3 (measured #3); electrophoresed #1, 2, 3; measured shells #1, 2, 3. (30) SC: McCormick County (GS-176; FMNH 214930): 13 live adults, 13 tissue sample—dissected #6, 7, 8 (measured #7; illustrated #6); electrophoresed #1, 2, 3, 6, 10; measured shells #4, 6, 11 (illustrated #H). (31) SC: Aiken County (GS-179; FMNH 214933): 11 live adults—dissected #A, B, C (measured #A); examined 3 partially everted penes; measured shells #A, B, C. (32) AL: Cleburne County (GS-180; FMNH 214935): 1 live adult—examined partially everted penis; measured shell.

Comparisons

Penis. *N. major* has the largest pilastral lappets—twice as many per unit length as *albolabris* (Table 4)—with the most convex, wavy surfaces. This species also has the largest wall pustules of the *albolabris* group (Table 4). In all other aspects it is similar to *N. albolabris,* and in fact much resembles an overgrown version of this species (compare Figs. 4 and 2d).

Shell. *N. major* has the least glossy shell with the relatively narrowest lip of both the *albolabris* and *alleni* groups (Table 7). Its striae are less dense than any of these taxa except *albolabris bogani,* in which the striae are much lower and less distinct (Table 7). The shells of *N. major* and *Mesodon normalis* are often sympatric and sometimes indistinguishable (Emberton, 1986).

Key characters

Penis: internal length ca 17 mm and relatively invariable; pilaster ca 1/10th as broad as the penis is long, and bearing 4–5 lappets per 2.6 mm; lappet surfaces wavy and very convex; verge 1/8th to 1/7th as long as the penis.

Shell: diameter 27–40 mm, depressed-globose, whorls 5 1/2–6; striae moderately raised, 16–34 per 2.6 mm on the 5th whorl; brown to dark brown; dull; whorls moderately expanding for the group; apertural lip relatively narrow for the group; basocolumellar node generally conspicuous; pre-apertural deflection moderate.

Remarks

Although the differences in penial morphology between *major* and *albolabris* (Figs. 4 and 2d) are arguably slight enough to denote only subspecific distinction, the available evidence supports Hubricht's recognition of *ma-*

jor as a full (sister) species. The penial difference is extremely consistent and uniform geographically, and although *albolabris* and *major* have never been found sympatric, their differences in penial size and sculpture are comparable to those between sympatric *albolabris* and *dentifera* (Fig. 2a, d). The shell differences between *major* and *albolabris* are distinct (Table 7) and disjunct, with no sign of clinal or hybridal intergradation. The electrophoretic difference is small (Table 2) but on the order of that found among other species pairs of the *albolabris* and *alleni* groups (Fig. 26, 27). There is a need for more fieldwork in Virginia to test for range overlap or intergradation.

Species Group *Neohelix alleni*
(Figs. 7, 6b, 30a–b, 32c–d; Tables 2, 4, 6, 7; Figs. 47, 49)

Key Characters

Penis: pilastral lappets approximately equal to the number of columns of wall pustules; pilaster moderately wide to narrow; wall columns with distinct pustules or locally smooth; wall pustules equal in size, or large except baso-laterally; verge moderate to minute, apical or dorsally subterminal; retractor-muscle origin close (less than 1/3rd the penial length) to the penial apex; vas deferens less than 2 1/2 times as long as the penis.

Neohelix alleni (Sampson, 1883)
(Figs. 3, 30a–b; Tables 2, 4, 6, 7; Figs. 47, 49)

Comparisons

Penis. *N. alleni* differs markedly from the *albolabris* group *(albolabris* and *major)* by its much shorter vas deferens, its retractor muscle attachment very close to the penial apex, its relatively short verge, and its flat- and smooth-surfaced, tightly appressed, densely packed pilastral lappets (Table 4, Fig. 3). Its differences from *N. solemi* are discussed under that species.

Shell. The only single character which distinguishes the shell of *alleni* from other species of the *albolabris* and *alleni* groups is its relatively faster whorl expansion rate (Table 7). It can also be separated from *albolabris* and *solemi* by its pronounced baso-columellar node (Fig. 30a), and from *major* by its glossier surface, yellower color, and denser striae (Table 7).

Key Characters

Penis: internal length 10–18 mm; pilaster ca 1/10th as broad as the penis is long, and bearing 15–18 lappets per 2.6 mm; lappet surfaces flat and smooth, lappets closely appressed; verge apical, ca 1/10th as long as the penis.

Shell: diameter 23–38 mm, depressed to depressed-globose, whorls 5–6; striae relatively low, 16–26 per 2.6 mm on the 5th whorl; yellow to yellow-brown; glossy; whorls rapidly expanding for the group; baso-columellar node large and conspicuous; preapertural deflection slight to very slight.

Neohelix alleni alleni (Sampson, 1883)
(Fig. 3a; Tables 2, 7; Figs. 47, 49)

Studied material

(1) IA: Lynn County (GS-17; FMNH 214908): 1 live adult, 1 tissue sample—dissected; electrophoresed. (2) IA: Jackson County (GS-18; FMNH 214909): 1 live adult, 1 tissue sample—dissected; electrophoresed. (3) AR: Crawford County (GS-90; FMNH 214910): 8 live adults, 8 tissue samples—dissected #4, 5, 6; electrophoresed #1, 3, 4, 5, 8; measured shells #1, 2, 8. (4) AR: Izard County (GS-97; FMNH 214911): 15 live adults, 15 tissue samples—dissected #11, 12, 13 (measured #13; illustrated #12); electrophoresed #1, 2, 4, 5, 6, 8. (5) AR: Izard County (GS-98; FMNH 214913): 4 live adults—illustrated shells #A, B (illustrated #B). (6) IA: Clayton County (FMNH 171135): 1 live adult—dissected (measured); illustrated shell. (7) AR: Izard County (FMNH 176221): 1 live adult—dissected; illustrated shell.

Comparison

This is the western (trans-Mississippian), typical, widespread subspecies of *alleni.* It differs from its eastern counterpart in its higher-spired, yellower, glossier, more densely striate, and generally smaller shell, with a slower whorl expansion rate, a less pronounced baso-columellar node, and a more pronounced pre-apertural deflection (Table 7).

Key characters

Shell: diameter 24–30 mm, depressed-globose, whorls 5–6; striae low, 16–22 per 2.6 mm on the 5th whorl; yellow to yellow-brown: glossy; whorl expansion rate low for the species; apertural lip wide for the species; basocolumellar node small for the species; preapertural deflection pronounced for the species.

Neohelix alleni fuscolabris (Pilsbry, 1903) (Fig. 3c; Tables 2, 7; Figs. 47, 49)

Studied material

(8) AL: Madison County (GS-20; FMNH 214): 14 live adults, 17 tissue samples—dissected #7, 11, 15; electrophoresed #1, 2, 4, 5, 7, 8, 9, 10, 11, 12, 13; illustrated shells #7, 11. (9) AL: Madison County (GS-101; FMNH 214): 4 live adults, 4 tissue samples—dissected #1, 2 (measured #2; illustrated #1); illustrated shell #1.

Comparison

This is the disjunct eastern subspecies of *alleni.* For shell differences, see comparative remarks under subspecies *alleni alleni* above.

Key characters

Shell: diameter 33–39 mm, depressed, whorls 5 1/2–6; striae moderately raised, 17–20 per 2.6 mm on the 5th whorl; brownish yellow; dull for the species; whorls rapidly expanding for the species; apertual lip relatively narrow for the species; basocolumellar large and pronounced for the species; preapertural deflection very slight for the species.

Neohelix solemi Emberton, new species (Figs. 6b, 32c–d; Tables 2, 4, 6, 7; Figs. 47, 49)

Synonymy

Helix albolabris var. *maritima* Pilsbry, 1890, Proc. Acad. Nat. Sci. Phila., p. 283, 3 figs. (shell, genitalia, radular teeth). Pilsbry, 1892, Nautilus 5: 142. Walker, 1906, Ill. Cat. Moll. Michigan, part I, p. 465, fig. 13. Cockerell, 1918, Nautilus 31: 108 (Ram Island, MA). Not *Helix maritima* Draparnaud, 1805.

Triodopsis albolabris form *traversensis* (Leach) Pilsbry, 1940, Land Moll. North Amer., pp. 836–839, Fig. 489 #9 (shell). Hackney, 1944, Nautilus, 58: 56 (Beaufort, NC). Jacobson, 1945, Nautilus, 59: 68 (Westchester County, NY). Alexander, 1947, Nautilus, 60: 97 (Cape May Point, NJ).

Triodopsis albolabris (Say) of Rehder, 1949, Nautilus, 62: 121 (Lake Waccamaw, Columbus County, NC); and, in part, of McCracken & Brussard, 1980, Evolution, 34: 92 ("Moriello Orchard", NY, and "Appledore Island", ME, populations).

Triodopsis albolabris albolabris (Say) plus *T. a. major* (Binney), in part, of Vagvolgyi, 1968, Bull. Mus. Comp. Zool., 136: 145 (northeastern Coastal Plain).

Triodopsis albolabris (Say) plus *T. major* (Binney), in part, of Hubricht (1985) (northeastern Coastal Plain).

Studied material (holotype and paratypes)

(33) NC: Catawba County (GS-32; FMNH 214936): 1 live adult—dissected (illustrated); illustrated shell (illustrated). (34) NC: Columbus County (GS-39; FMNH 214937): 1 live adult—dissected; illustrated shell. (35) SC: Williamsburg County (GS-41; FMNH 214939): 1 live adult—dissected (measured); illustrated shell. (36) NC: Columbus County (GS-164; FMNH 214941): 3 live adults—dissected #A, B, C (measured #B); illustrated shells #A, B, C. (37) NC: Columbus County (GS-165; FMNH 214942): 1 live adult—dissected. (38) NJ: Cape May County (GS-208; FMNH 214943): 1 live adult, 3 tissue samples—dissected #1, electrophoresed #1, 2, 3. (39) NC: Scotland County (SC-101; FMNH 214945) (HOLOTYPES): 3 live adults, 7 tissue samples—dissected #5, 6, 7. (40) NC: Scotland County (SC-103; FMNH 214946): 3 live adults, 3 tissue samples—dissected #1, 2, 3. (41) NC: Wake County (SC-138; FMNH 214947): 1 live adult, 1 tissue sample—dissected. (42) NC: New Hanover County (SC-277; FMNH 214948): 5 live adults—dissected #A, B, C, D, E. (43) NJ: Cape May County (ANSP 63869-A2432): 6 live adults—dissected #A. (44) NJ: Cape May County (ANSP 72764-A2431): 13 live adults—dissected #A, B. (46) NY: Westchester County (ANSP 181296-A2410): 1 live adult—dissected.

Comparisons

Penis. This species is unique within *Neohelix* in having a (dorsally) subterminal pore, a

ventral pilaster, a greatly reduced dorsal pilaster, and a greatly elongated basal penis (Fig. 6b).

Shell. The only shell character distinguishing *N. solemi* from other members of the *albolabris* and *alleni* groups is its relatively dark brown color (Table 7), but there is overlap in color (Table 6), so this is not reliable for identification. Practically speaking, *solemi* need only be distinguished from *N. albolabris* and *major,* both of whose ranges appear to be parapatric to it (Fig. 47). It usually differs from *N. albolabris* by its narrower apertural lip, slightly higher spire, and slightly slower whorl expansion rate; it differs from *major* by the weakness of its baso-columellar node and by its slightly higher spire, slower whorl expansion rate, glossier surface, and slightly denser striae (Table 7). These differences are based on statistical comparisons of small samples however, and should be used only as guidelines, not as absolutes, for identification. The only reliable way to distinguish *solemi* from *albolabris* or *major* is by dissection, as shown by two misclassified shells in the discriminant analysis, discussed above.

Key characters

Penis: internal length 12–17 mm; pilaster 1/50th to 1/25th as broad as the penis is long, and bearing 14–15 lappets per 2.6 mm; pilaster abbreviated in length by the subterminal pore position; pore dorsally subterminal, mounted on a thick, fleshy pedestal; verge 1/100th to 1/20th as long as the penis; basal penis long, with 1/2 or more of the total penis length lying between the vaginal opening and the base of the sheath.

Shell: diameter 24–35 mm, depressed-globose, whorls 5–6; striae moderately raised, 16–21 per 2.6 mm on the 5th whorl; dark brown; moderately dull; whorl expansion rate relatively slow; relative apertural lip width relatively low; pre-apertural deflection moderate.

Remarks

Pilsbry (1940: 839) noticed one of the anatomical distinctions of this species—its short vas deferens—but was led by shell similarities to synonomyze it with *N. albolabris traversensis* (Leach) of Traverse City, Michigan, and nearby localities. Except for this Michigan disjunct, he reported its range (based on shell material) as Coastal Plain Maine to North Carolina; this conforms well with distributional findings based on anatomical studies (Fig. 47), which further extend the range into Coastal Plain South Carolina. No material north of Westchester County, New York has yet been anatomically verified, to my knowledge, but electrophoretic and conchological comparisons have convinced me (Emberton, McCracken, & Wooden, in preparation) that *solemi* occurs in Ulster County, New York (FMNH 214952) and York County, Maine (FMNH 214950). Although I have not yet dissected topotypic *N. albolabris traversensis* (Leach), I have little doubt about its being a different species because of its extreme western disjunction from the known range of *solemi* (Fig. 47). The discriminant analysis, as discussed above, has shown that the *albolabris albolabris* shell can be mistaken for that of *solemi.*

This species is named for Dr. Alan Solem, Curator and Head, Division of Invertebrates, Field Museum of Natural History, Chicago, eminent terrestrial malacologist and mentor.

APPENDIX C. SYSTEMATIC REVIEW OF THE SUPRASPECIFIC TAXA OF THE EASTERN AMERICAN TRIODOPSINAE

The eastern American triodopsines differ from all other polygyrids in having a single dorsal pilaster in the upper penis. As in the polygyrid genera *Vespericola, Cryptomastix,* and *Allogona,* they have a penial sheath, a retentor muscle, an upper penis, and the penial retractor muscle attaches to the vas deferens (Fig. 11), but they differ from these other genera in the following ways. First, eastern triodopsines lack both epiphallus and flagellum, both of which are present, although not always conspicuous, in *Vespericola, Cryptomastix,* and *Allogona.* Second, the basal penis of eastern triodopsines is never wider than, nor longer than, the upper penis and never contains any lobes, flaps, or nonrandom folds; this differentiates them from both *Cryptomastix* and *Allogona.* Third, when eastern triodopsines have a verge, it is always flat with a subterminal pore and terminal papillae; the verge of *Vespericola* differs in being roundly conical with a simple terminal pore. Fourth, when the shells of eastern triodopsines are large and toothless, they are also always imperforate, and therefore are readily distinguishable from the widely umbilicate large shells of *Allogona* (Fig. 46).

Therefore, any snail which (a) is east of the 100th meridian, (b) has a polygyrid shell which is not *Allogona profunda* (see Fig. 46), and (c) has a penial sheath (and retentor muscle)——is a triodopsine. The penial characters are essential for identification because on shell characters alone many eastern triodopsines are easily confused or even indistinguishable from the geographically overlapping polygyrine genus *Mesodon* (see Pilsbry, 1940; Solem, 1976; Emberton, 1986). Anatomically these two lineages are readily distinguishable by the external aspect of the uneverted penis: *Mesodon* lacks the penial sheath, the retentor muscle, and the thickened spermathecal duct of triodopsines, and its penial retractor muscle inserts on the apex of the penis rather than on the vas deferens. In addition, the thick spermathecal duct of triodopsines distinguishes them from *Mesodon.* (An easy, though destructive way to identify an adult polygyrid as a *Mesodon* or an eastern-triodopsine in the field is to lightly step on it: the penis can then be diagnosed.)

Genus *Webbhelix* Emberton, new genus
(Figs. 6a, 32a–b; Table 2; Fig. 49)

Comparisons

Webbhelix is unique among triodopsines in having the dorsal pilaster covered with uniform pustules equal in size to the wall pustules (Fig. 6a). It is also the only triodopsine known to have spiral color bands on the shell (Fig. 32b), although these are not always present.

Key characters

Penis: pilaster approximately 3/4-length, abruptly truncated basally, and covered with uniform sharply-pointed pustules equal in size to wall pustules; wall pustules arranged in approximately 25 contiguous longitudinal columns and partially fused along their columns basally; verge large, with two broad and prominent terminal papillae, and smooth-surfaced.

Shell: diameter 14.5–32 mm, depressed-globose, whorls 5 1/2–6; imperforate; thin, thin-lipped; usually marked with reddish-brown color bands.

Discussion

This genus, which occupies a basal phylogenetic position, is named for Dr. Glenn R. Webb, recently retired from Kutztown University, Pennsylvania, whose forty years of dedicated research and publishing are so basic to our understanding not only of eastern triodopsines but of many other North American land pulmonates.

Webbhelix multilineata (Say, 1821)
(Figs. 6a; 32a–b; 32a–b; Table 2; Fig. 49)

Studied material

(1) IL: Marshall County (GS 127; FMNH 214848): 2 live adults, 2 tissue samples—dissected #2 (illustrated #2); electrophoresed #1; illustrated shell #2. (2) IL: Kane-Cook Counties: (GS 207; FMNH 214849): ca 11 live adults, 15 tissue samples—dissected #1, 5, A; electrophoresed #1, 3, 5. (3) IL: Calhoun County: (Hubricht 48600) ca 15 live adults—dissected #A, B, C.

Published anatomies

(1) Binney 1851, Plate VIII. (2) Webb 1948, Figs. 2, 2a. (3) Webb 1952, Plate 5, Figs. 1–8. (4) Webb 1954, Plate 10, Fig. 10.

Discussion

Webb (1952: 8) elevated Pilsbry's (1940: 850) form *chadwicki* (Ferriss, 1907) to a full species, but both Vagvolgyi (1968) and Hubricht (1985) synonymized it with *multilineata.*

Genus *Neohelix* von Ihering, 1892
(Figs. 2–5, 6b, 29–31, 32c–d; Table 2; Fig. 49)

Comparisons

Neohelix is the only genus of eastern triodosines which has pilastral lappets (Figs. 2b, e; 5c, f). It is the only genus besides *Webbhelix* which has its wall pustules arranged in 25–35 contiguous, longitudinal columns; which has a large verge, although its verge size varies; and which has a large, toothless, imperforate shell. Its shell and apertural lip seem to be always thicker than in *Webbhelix* and its shell is never banded as in *Webbhelix. Neohelix* also differs from

Xolotrema and *Triodopsis* in never having a ventrally subterminal pore and in never having either a palatal or a basal apertural barrier. It differs from *Triodopsis* in having a closed umbilicus. Generally, any eastern triodopsine with a shell which is imperforate, smooth-lipped, and unbanded is a *Neohelix.*

Key characters

Penis: dorsal pilaster full-length, smoothly terminating basally, and armed with lappets, lappet number either approximately the same or approximately twice the number of columns of wall pustules, pilaster rarely vestigial; wall pustules arranged in 25–35 contiguous, longitudinal columns, and either uniform in size or larger basally; verge large to vestigial, always with a corded surface and thin terminal papillae; pore terminal or, rarely, dorsally subterminal.

Species Group *Neohelix albolabris*

See Appendix B.

Species group *Neohelix alleni*

See Appendix B.

Species group *Neohelix dentifera*
(Figs. 2a–c, 5, 29a–b, 31; Table 2; Fig. 49)

Comparisons

Penis. The *dentifera* group differs from other *Neohelix* in the doubled number of its pilastral lappets (Figs. 2a, 5a, d) and the incomplete lateral fusion of the lappets' component pustules (Figs. 2b, 5c, f), as well as in the enlargement of its basal-most wall pustules (Figs. 2a, 5a, d).

Shell. The *dentifera* group's shell is much more depressed than in other *Neohelix* (Figs. 29b, 31b, d). The only species of this group which occurs east of the Mississippi, *dentifera,* is readily distinguished from all other eastern *Neohelix* by its well developed parietal tooth and wide apertural lip (Fig. 31a); the parietal tooth which occurs rarely in *albolabris* (e.g. Pilsbry, 1940, fig. 489 #8) is always much weaker than *dentifera's.*

Key characters

Penis: pilastral lappets equal in number to approximately twice the number of columns of body wall pustules; pilastral pustules comprising the lappets only partially fused laterally; basal-most wall pustules large; verge large, terminal.

Shell: diameter 16–30 mm, depressed, whorls 4 1/2–5 1/2; parietal tooth absent or strong; lip smooth or with a small bump suggesting a basal tooth or lamella (Fig. 29a); lip narrow to broad; striae weak to moderately strong.

Species subgroup *Neohelix dentifera*
(Figs. 2a–c, 29a–b; Table 2; Fig. 49)

Key characters

Penis: basal-most 2–3 layers of wall pustules enlarged.

Shell: diameter 20–30 mm, whorls 5–5 1/2; parietal tooth strongly developed; apertural lip very thick and wide; basal lip sometimes with a bump suggesting a tooth or lamella; striae moderately strong.

Neohelix dentifera (Binney, 1837)
(Figs. 2a–c, 29a–b; Table 2; Fig. 49)

Studied material

(1) WV: Preston County (GS-130; FMNH 214809): 20 live adults, 20 tissue samples—dissected #2, 7, 14; electrophoresed #1, 2, 5, 15, 16. (2) WV: Pendleton County (GS-134; FMNH 214810): 10 live adults, 10 tissue samples—dissected #1, 4, 8 (illustrated #8); electrophoresed #5; illustrated shell #8.

Species subgroup *Neohelix divesta*
(Figs. 5d, 21; Table 2; Fig. 49)

Key characters

Penis: Basal-most 8–15 layers of wall pustules enlarged.

Shell: Diameter 14–18 mm; whorls 4 1/2–5; parietal tooth always absent; apertural lip evenly narrow and always perfectly smooth internally; striae weak.

Neohelix divesta (Gould, 1848)
(Figs. 5d–f, 31c–d; Table 2; Fig. 49)

Studied material

(1) AR: Crawford County (GS-90; FMNH 214813): 1 live adult, 2 tissue samples—dissected #1, 7, 8, 10 (illustrated #1);

electrophoresed #1, 2; illustrated shell #A (FMNH 214815). (2) AR: Logan county (GS-95; FMNH 214814): ca 4 live adults, 19 tissue samples—electrophoresed #3, 9, 13, 16, 18.

Neohelix lioderma (Pilsbry, 1902)
(Figs. 5a–b, 31a–b; Table 2; Fig. 49)

Studied material

(1) OK: Tulsa County (GS-82; FMNH 214844): 9 live adults, 15 tissue sample—dissected #9, A, B, C (illustrated #A); electrophoresed #1, 5, 6, 7, 9, 10, 12, 13, 14, 15; illustrated shell #A.

Remarks

N. lioderma was originally described as subspecies of the polygyrine *Mesodon indianorum* (see Pilsbry, 1940). It is obviously a very recently derived diminutive of *divesta,* with a restricted, relict range peripheral to that of *divesta* (Fig. 49).

Genus *Xolotrema* (Rafinesque, 1819)
(Figs. 7, 8, 33, 34; Table 2; Fig. 49)

Comparisons

Penis. *Xolotrema* differs from the other three genera of eastern triodopsines by the gradual dorsal enlargement of its pustules (wall-to-pilaster); its Type 3 chevron; and its very small, apical or ventrally subterminal—never dorsally subterminal—verge.

Shell. Conchologically, *Xolotrema* is unique among eastern triodopsines in its long, smoothly curved parietal tooth which never abruptly changes height; its long, blade-like basal lamella; and its basally-pointing palatal tooth (Figs. 33a, c, e; 34a, c). It includes the only triodopsines with an angular (Figs. 33d, 34b, d) or keeled (Fig. 33f) periphery, or with hair-like periostracal processes (Fig. 33a, b). *Xolotrema* can always be distinguished from *Webbhelix* and *Neohelix* by its possession of a palatal tooth and a basal lamella, and from *Triodopsis* by the complete coverage of its umbilicus by an extension of the reflected apertural lip in the adult.

Key characters

Penis: pustules gradually enlarging dorsally, largest on the pilaster; pilastral pustules arranged either in a single column of abutting cubes or in 5 broad, nested A-shapes; wall pustules arranged in tapered, slightly separated columns all merging ventrally into 6–10 U-shapes; verge small, bearing 4–6 narrow terminal papillae; verge either terminal or ventrally subterminal and apically directed; everted penis either tubular or shaped like an everted pear; ventral sperm groove present or absent; sheath either covering entire upper (uneverted) penis or covering less than half.

Shell: diameter 8–27 mm, depressed, whorls 4 1/2–5 1/2; periphery keeled, angular, or rounded; parietal tooth long, high-standing, gently arched, smoothly decreasing in height toward the umbilicus; basal barrier in the form of a long, blade-like lamella; palatal tooth very strong to weak, pointing downward toward the basal lamella; striae either very to moderately strong, or weak and masked by dense hair-like periostracal processes.

Species group *Xolotrema fosteri*
(Figs. 8, 34; Table 2; Fig. 49)

Key characters

Penis: pilastral pustules a single column of abutting cubes; verge terminal, bearing 6 terminal papillae; everted penis tubular; ventral sperm groove present; sheath entirely covering uneverted upper penis.

Shell: diameter 14–20, whorls 4 1/2–5 1/2; periphery slightly angled or with an angled shoulder; palatal tooth moderate to weak; striae moderately strong to strong; surface free of pustules or hair-like processes.

Xolotrema fosteri (F. C. Baker, 1932)
(Figs. 8a, 34a–b; Table 2; Fig. 49)

Studied material

(1) KY: Hancock County (H-22; FMNH 214817): 1 live adult—dissected #A, B, C, D, E (illustrated #A); illustrated shell #15. (2) KY: Hancock County (GS-15; FMNH 214819): 24 live adults, 24 tissue samples—dissected #19; electrophoresed #12, 13, 14, 15, 16, 17, 18, 19, 21, 22.

Xolotrema occidentalis (Pilsbry & Ferriss, 1907)
(Figs. 8b–c, 34c–d; Table 2; Fig. 49)

Studied material

(1) AR: Independence County (GS-99; FMNH 214855): 1 live adult, 10 tissue samples—electrophoresed #2, 3. (2) AR: Independence County (GS-100; FMNH 214856): 5 live adults, 10 tissue samples—dissected #5 (illustrated #5); electrophoresed #1, 2, 3, 4, 10; illustrated shell #5.

Species group *Xolotrema denotata*
(Figs. 7, 33; Table 2; Fig. 49)

Key characters

Penis: pilastral pustules in 5 broad, nested A-shapes; verge subterminal, apically directed, bearing 4 terminal papillae; everted penis shaped like an inverted pear; ventral sperm groove absent; sheath covering less than half the uneverted upper penis.

Shell: diameter 17–26 mm, whorls 5–6; periphery keeled, to angled, to rounded; palatal tooth very strong; striae either very to moderately strong, or weak and masked by sense hair-like periostracal processes.

Xolotrema denotata (Férussac, 1821)
(Figs. 7a–b, 33a–b; Table 2; Fig. 49)

Studied material

(1) IN: Jefferson County (GS-14; FMNH 214805): 0 live adults, 2 tissue samples—electrophoresed #1, 2. (2) KY: Fayette County (GS-112; FMNH 214806): 7 live adults, 13 tissue samples—dissected #1, 2, 6 (illustrated #6); electrophoresed #1, 2, 5, 6, 7, 8, 10, 11, 13; illustrated shell #1.

Xolotrema obstricta (Say, 1821)
(Figs. 7c–d, 33e–f; Table 2; Fig. 49)

Studied material

(1) KY: Henderson County (GS-16; FMNH 214852): 1 live adult, 1 tissue sample—electrophoresed #1; illustrated shell #1. (2) AL: Madison County (GS-20; FMNH 214853): 1 live adult, 1 tissue sample—electrophoresed #1. (3) KY: Edmonson County (GS-125; FMNH 214854): 15 live adults, 16 tissue samples—dissected #1, 9 (illustrated #9); electrophoresed #1, 3, 4, 6, 10.

Xolotrema caroliniensis (Lea, 1834)
(Figs. 7e, 33c–d; Table 2; Fig. 49)

Studied material

(1) AL: DeKalb-Marshall Counties (GS-184; FMNH 214): 1 subadult, 1 tissue sample—electrophoresed #1. (2) TN: Franklin County (FMNH 171142): 5 live adults—dissected #A, B (illustrated #A); illustrated shell #B.

Genus *Triodopsis* (Rafinesque, 1819)
(Figs. 9–18, 35–45; Table 2; Fig. 49)

Comparisons

Penis. *Triodopsis* differs from the other three genera of eastern triodopsines by the abruptly larger pustules on its pilaster.

Shell. *Triodopsis* is unique among eastern American triodopsines in having an open umbilicus and a distinct, non-lamellar basal tooth.

Key characters

Penis: pilastral pustules abruptly larger than wall pustules; pilastral pustules either unfused, fused into nesting horseshoe shapes, fused into two columns of interdigitating rectangular box shapes, fused into grossly irregular elements, fused into a solid apical mass bearing three to four tiers of long and sharp spurs, or fused into irregular polygons bearing short and blunt spurs; wall-pustular columns separated and either radiating from the pore, 15–20 (or rarely 8–10) in number, and unmerging or incompletely merging basally; or completely merging ventrally to form either 10–12 obtuse V-shapes or 5–7 acute V-shapes; wall-pustular columns with pustules distinct, with pustules partially fused, or smooth with no sign of pustules; verge absent; pore terminal or ventrally subterminal; penis short, to long, to extremely long and thread-like; erectile, fleshy peduncle below the pore large, small, or absent.

Shell: diameter 8–27 mm, depressed-globose to depressed, whorls 4 1/2–6 1/2; umbilicus wide and open to minute and creviced; parietal tooth prominent, varying from straight to abruptly angled up to about 120 degrees, from uniformly high-standing to abruptly changing in height; basal tooth weak (rarely absent) to pronounced, varying from peg-like to tapered, from simple to buttressed to bidentate, and from marginal to deeply re-

cessed; palatal tooth pointing toward the umbilicus, weak (rarely absent) to pronounced, varying from broad to narrow, from squared to tapered, from simple to buttressed, and from marginal to deeply recessed; striae very weak to very strong.

Species group *Triodopsis vulgata*
(Figs. 9, 10, 35, 36; Table 2; Fig. 49)

Key characters

Penis: pilastral pustules unfused or fused into nesting horseshoe shapes; wall pustular columns 15–20, radiating from the pore, unmerging or partially merging basally, and either with distinct pustules or nearly smooth; pore ventrally subterminal, about 1/5-way from the apex, everted penis shaped like an angled baseball bat.

Shell: diameter 10–19.5 mm, depressed, whorls 4 1/2–6; aperture deeply dished; apertural periphery with a squared-off appearance; parietal tooth straight, broadly wedge-like, and symmetrical or slightly angled and tapered toward the umbilicus; basal tooth peg-like, marginal; palatal tooth broad, squared, recessed; striae moderate to very strong.

Species subgroup *Triodopsis vulgata*
(Figs. 9a, c; 35a, b, e, f; Table 2; Fig. 49)

Key characters

Penis: pilastral pustules unfused; wall pustular columns never merging.

Shell: parietal tooth slightly angled and tapered toward the umbilicus.

Triodopsis vulgata Pilsbry, 1940
(Figs. 9a, 35a–b; Table 2; Fig. 49)

Studied material

(1) TN: Morgan County (GS-109; FMNH 214883): 20 live adults, 18 tissue samples—dissected #2, 3. (2) KY: Fayette County (GS-112; FMNH 214884): 7 live adults, 8 tissue samples—dissected #1 (illustrated #1); electrophoresed #1, 2, 3, 4, 6, 7, 8; illustrated shell #A. (3) KY: Harlan County (GS-119; FMNH 214885): 9 live adults, 11 tissue samples—dissected #1, 2, 3, 4; electrophoresed #2, 3, 6.

Triodopsis claibornensis Lutz, 1950
(Figs. 9c, 35e–f; Table 2; Fig. 49)

Studied material

(1) TN: Claiborne County (GS-117; FMNH 214800): 22 live adults, 22 tissue samples—dissected #5, 18 (illustrated #18); electrophoresed #1, 5, 16, 20; illustrated shell #A.

Species subgroup *Triodopsis fraudulenta*
(Figs. 9b, 10, 35c–d, 36; Table 2; Fig. 49)

Key characters

Penis: pilastral pustules fused into nesting horseshoe shapes; wall-pustular columns unmerging or partially merging basally, with distinct pustules or nearly smooth.

Shell: parietal tooth straight, broadly wedge-like, and symmetric.

Triodopsis fraudulenta (Pilsbry, 1894)
(Figs. 10, 36; Table 2; Fig. 49)

Studied material

(1) WV: Greenbrier County (GS-139; FMNH 214822): ca 5 live adults, 11 tissue samples—dissected #6, 8 (illustrated #6); electrophoresed #2, 4, 5, 6, 7, 11; illustrated shell #A.

Triodopsis picea Hubricht, 1958
(FIgs. 9b, 35c–d; Table 2; Fig. 49)

Studied material

(1) WV: Pendleton County (GS-134; FMNH 214860): 20 live adults, 20 tissue samples—dissected #4, 14 (illustrated #14); electrophoresed #1, 5, 9, 11, 17; illustrated shell #15.

Species group *Triodopsis platysayoides*
(Figs. 12, 37; Table 2; Fig. 49)

Key characters

Penis: pilastral pustules fused into two columns of interdigitating rectangular box shapes; wall-pustular columns completely merging ventrally to form 10–12 obtuse V-shapes; pore terminal.

Shell: diameter 27 mm; spire nearly flat; umbilicus very broad and open; parietal tooth short, nearly straight, high-standing, symmet-

rical, and scooped internally; basal tooth very low, with broadly tapered sides; palatal tooth absent.

Triodopsis platysayoides (Brooks, 1933)
(Figs. 12, 37; Table 2; Fig. 49)

Studied material

(1) WV: Preston County (SC-273; FMNH 214861): 2 live adults, 5 tissue samples (collected under U.S. Dept. Interior Fish & Wildlife Permit # PRT-670226 and W. Va. Dept. Nat. Res. Scientific Collecting Permit No. 17, 1984, both to the author)—dissected #1, 2 (illustrated #1); electrophoresed #1, 3, 4, 5; illustrated shell #2. (2) WV: Preston County (Hubricht 11860): 1 live adult—examined dissection done by Solem (1976).

Species group *Triodopsis burchi*
(Figs. 11a, 37a–b, Table 2; Fig. 49)

Key characters

Penis: pilastral pustules fused into grossly irregular elements irregular in size and shape; wall-pustular columns ca 15, radiating from the pore, unmerging, and semi-smooth; pore terminal.

Shell: diameter 8–17 mm; spire extremely low; parietal tooth as in the *platysayoides* group; palatal tooth high, tiny, triangularly pointed, and marginal.

Triodopsis burchi Hubricht, 1950
(Figs. 11a, 37a–b; Table 2; Fig. 49)

Studied material

(1) VA: Patrick County (GS-143; FMNH 214797): ca 10 live adults, 14 tissue samples—dissected #3, 5, 12 (illustrated #3); electrophoresed #3, 4, 7, 9, 11, 14; illustrated shell #10.

Species group *Triodopsis tennesseensis*
(Figs. 11b–d, 37c–f; Table 2; Fig. 49)

Key characters

Penis: pilastral pustules fused into a solid apical mass bearing three to four tiers of long, sharp spurs; wall-pustular columns as in the *burchi* group, except completely smooth.

Shell: diameter 9–25 mm; spire low; apertural teeth as in the *burchi* group; striae either very strong or very weak.

Triodopsis tennesseensis (Walker & Pilsbry, 1902)
(Figs. 11b–c, 37c–d; Table 2; Fig. 49)

Studied material

(1) KY: Fayette County (GS-112; FMNH 214864): 18 live adults, 18 tissue samples—dissected #13, 14, 15 (illustrated #15); electrophoresed #2, 5, 18; illustrated shell #7. (2) KY: Pulaski County (GS-124; FMNH 214865): 7 live adults, 12 tissue samples—electrophoresed #1, 6.

Triodopsis complanata (Pilsbry, 1898)
(Figs. 11d, 37e–f; Table 2; Fig. 49)

Studied material

(1) KY: Pulaski County (GS-13; FMNH 214802): 0 live adults, 1 tissue samples—electrophoresed #1, 2. (2) KY: Pulaski County (Hubricht 17932): ca 9 live adults (live into isopropynol)—dissected #A, B, C (illustrated #C); illustrated shell #A.

Species group *Triodopsis rugosa*
(Figs. 18b, 45c–d; Table 2; Fig. 49)

Key characters

Penis: pilaster ca 2/3-length and proximally tapered; pilastral pustules fused to form irregular polygons each bearing 1–3 short, blunt spurs; wall-pustular columns either ca 15 or ca 9, partially fused basally, semi-smooth; pore terminal.

Shell: diameter 8–11 mm; depressed; umbilicus moderate; parietal tooth as in the *vulgata* group; basal and palatal teeth peglike, strongly buttressed, slightly recessed; striae very strong, modertaely to widely spaced.

Triodopsis rugosa Brooks & Macmillan, 1940
(Fig. 49)

Studied material

(1) WV: Logan County (SC-278; FMNH 214888): 6 live adults, 11 tissue samples—dissected #1, 2.

Triodopsis fulciden Hubricht, 1952
(Figs. 18b, 45c–d; Table 2; Fig. 49)

Studied material

(1) NC: Burke County (GS-35; FMNH 214823): 5 live adults, 5 tissue samples—dissected #3 (illustrated #3); electrophoresed #2, 3; illustrated shell #A.

Species group *Triodopsis cragini*
(Figs. 13, 39; Table 2; Fig. 49)

Key characters

Penis: penis extremely long and thread-like; pilaster as in the *rugosa* group; wall-pustular pilaster columns completely fused ventrally into 5–7 acute V-shapes.

Shell: diameter 8.5–14.5 mm; depressed-globose; umbilicus small; parietal tooth slightly to pronouncedly scooped externally, umbilicad extension moderate to absent; basal tooth with an umbilicad extension varying from weak to equal in size to the basal tooth itself, and slightly to deeply recessed; basal lip bearing a weak to strong convex ridge; palatal tooth broad, rounded, and varying from moderately sized and recessed to very large and deeply recessed; striae weak to strong.

Remarks

The shells of the three species seem to form a continuum from least to most derived in the order *cragini, vultuosa, henriettae,* showing an increasing overgrowth of the apertural lip and dentition. This hypothesis is supported by the electrophoretically more primitive position of *cragini* in the Wagner-2 Tree (Fig. 27) and as depicted in the Consensus Tree (Fig. 28).

Triodopsis cragini Call, 1886
(Figs. 13b, 39c–d; Table 2; Fig. 49)

Studied material

(1) TX: Polk County (GS-73; FMNH 214803): 20 live dults, 20 tissue samples—dissected #3, 18 (illustrated #18); electrophoresed #1, 2, 4, 8, 10, 14; measured shell #2. (2) TX: Henderson County (GS-79; FMNH 214804): 7 live adults, 7 tissue samples—electrophoresed #2, 3, 6.

Triodopsis vultuosa (Gould, 1848)
(Figs. 13a, 39a–b; Table 2; Fig. 49)

Studied material

(1) TX: Walker County (GS-71; FMNH 214887): 18 live adults, 15 tissue samples—dissected #A, B (illustrated #A); electrophoresed #1, 9, 11; illustrated shell #7. (2) TX: Cherokee County (GS-78; FMNH uncat.): ? live adults, 11 tissue samples—electrophoresed #1, 6. (3) TX: Jefferson County (GS-208?; FMNH uncat.): ? live adults, ? tissue samples—electrophoresed #1, 2.

Triodopsis henriettae (Mazÿck, 1877)
(Figs. 13c, 39e–f; Table 2; Fig. 49)

Studied material

(1) TX: Houston County (GS-76; FMNH 214824): 2 live adults, 2 tissue samples—dissected #1, 2 (illustrated #2); electrophoresed #1, 2; illustrated shell #2.

Species group *Triodopsis tridentata*
(Figs. 14a–b, 14, 16, 17, 40, 42, 43, 44; Table 2; Fig. 49)

Key characters

Penis: penis length moderate; pilaster as in the *rugosa* and *cragini* groups; pore ventrally subterminal, ca 1/4-way from the apex; everted penis mace-shaped; moderate-sized peduncle beneath pore.

Shell: diameter 8–15 mm; depressed-globose to depressed; whorls 4 1/2–6 1/2; umbilicus moderate to minute; parietal tooth variable, ranging from that of the *vulgata* and *fraudulenta* groups, to that of the *burchi* and *tennesseensis* groups, to that of the *cragini* group with a more pronounced umiblicad extension, to a form superficially resembling that of the *denotata* group of *Xolotrema;* basal tooth marginal and variable, covering much of the range of shapes found in the *vulgata, rugosa,* and *cragini* groups, and rarely absent entirely; palatal tooth marginal and supraperipheral, to moderately recessed and subperipheral, and either peg-like (and buttressed or unbuttressed), or as in the *cragini* group (and buttressed or unbuttressed), or rarely absent; striae very weak to very strong.

Species subgroup *Triodopsis tridentata* (Figs. 14a–b, 40; Table 2; Fig. 49)

Key characters

Shell: diameter 12–15 mm; depressed; whorls 4 1/2–5 1/2; umbilicus moderate; parietal tooth either as in the *rugosa* group or as in the *burchi* or *tennesseensis* group; basal and parietal teeth as in the *rugosa* group, except either buttressed or unbuttressed (or rarely absent altogether), and with the palatal tooth marginal; striae very strong.

Triodopsis tridentata (Say, 1816) (Figs. 14a, 40a–b; Table 2; Fig. 49)

Studied material

(1) TN: Blount County (GS-8; FMNH 214866): 1 live adult, 1 tissue sample—electrophoresed #1. (2) TN: Blount County (GS-9; FMNH uncat.): ? live adults, 9 tissue samples—electrophoresed #1, 2, 3, 4, 5, 6, 7, 8, 9. (3) NC: Haywood County (GS-10; FMNH 214867): ca 10 live adults, 10 tissue samples—electrophoresed #6. (4) WV: Pendleton County (GS-134; FMNH 214875): 10 live adults, 10 tissue samples—dissected #3. (5) WV: Pocahontas County (GS-135; FMNH 214876): 4 live adults, 5 tissue samples—dissected #2 (illustrated #2); electrophoresed #1 2, 3, 4; illustrated shell #4. (6) KY: Harlan County (GS-119; FMNH 214872): 1 live adult, 1 tissue sample—dissected #1. (7) KY: Edmonson County (GS-125; FMNH 214873): 2 live adults, 2 tissue samples—dissected #1, 2. (8) WV: Preston County (GS-126; FMNH 214874): 15 live adults, 15 tissue samples—dissected #15. (9) NC: Avery County (GS-153; FMNH 214878): 10 live adults, 10 tissue samples—electrophoresed #2, 5, 7. (10) OH: Athens County: Site IV-1 (FMNH 209209): 10 live adults—dissected #C, D. (11) OH: Athens County: Site III-3 (FMNH 209536); 5 live adults—dissected #C. (12) Locality unknown (FMNH 171254): ? live adults—dissected #A.

Triodopsis anteridon (Pilsbry, 1940) (Figs. 14b, 40c–d; Table 2; Fig. 49)

Studied material

(1) KY: Harlan County (GS-121; FMNH 214793): 21 live adults, 21 tissue samples—dissected #13, 14; electrophoresed #6, 7, 16. (2) WV: Boone County (GS-142; FMNH 214796): 20 live adults, 20 tissue samples—dissected #18 (illustrated #18); electrophoresed #3, 10; illustrated shell #19.

Species group *Triodopsis fallax* (Figs. 15, 16, 17, 42, 43, 44; Table 2; Fig. 49)

Key characters

Shell: diameter 8–14 mm; depressed-globose; whorls 4 1/2–6 1/2; umbilicus moderate to minute; parietal tooth as in the *cragini* group, but with a more pronounced, angled umbilicad extension; apertural lip teeth as in the *cragini* group, but with the basal tooth marginal more strongly buttressed, the palatal tooth only slightly recessed; striae moderate to strong.

Remarks

The phylogeny of the *fallax* group is discussed in Appendix D. For species diagnoses see Grimm (1975), except for *palustris,* for which see Hubricht (1958).

Species subgroup *Triodopsis fallax* (Figs. 15b–c, 16b, 17, 42c–f, 43c–d, 44a–d; Table 2; Fig. 49)

Key characters

Shell: whorls 4.5–5.0, lip edge generally sharp, apertural teeth relatively indistinct; luster dull to very shiny.

Triodopsis fallax (Say, 1825) (Figs. 17a, 44a–b; Fig. 49)

Studied material

(1) NC: Richmond County (Hubricht 10209): 6 live adults (dropped live into isopropynol)—dissected #A, B, C (illustrated #C); measured shell #A.

Triodopsis messana Hubricht, 1952 (Figs. 16b, 43c–d; Table 2; Fig. 49)

Studied material

(1) NC: Columbus County (GS-163; FMNH 214846): ca 5 live adults, 10 tissue samples—dissected #1, 5, 6 (illustrated #6); electrophoresed #1, 7, 8, 9; illustrated shell #A.

Triodopsis palustris Hubricht, 1958
(Figs. 15b, 42c–d; Table 2; Fig. 49)

Studied material

(1) SC: Williamsburg County (GS-41; FMNH 214857): ca 5 live adults, 15 tissue samples—dissected #4, 5, 15 (illustrated #15); electrophoresed #5, 8, 10, 11, 15; illustrated shell #1. (2) GA: Wayne County (GS-49; FMNH 214858): ca 6 live adults, 15 tissue samples—electrophoresed #4, 9.

Triodopsis obsoleta (Pilsbry, 1894)
(Figs. 15c, 42e–f; Fig. 49)

Studied material

(1) NC: Chowan County (Hubricht 10300): 7 live adults (dropped live into isopropanol)—dissected #A, B, C (illustrated #C).

Triodopsis soelneri (Henderson, 1907)
(Figs. 17b, 44c–d; Fig. 49)

Studied material

(1) NC: New Hanover County (ANSP A2318): 3 live adults—dissected #A, B, C (illustrated #B). (2) NC: Columbus County (FMNH 159040): shells only-illustrated shell #A.

Species subgroup *Triodopsis alabamensis*
(Figs. 15a, 16a, c, 42a–b, 43a–b, e–f; Table 2; Fig. 49)

Key characters

Shell: whorls 4 1/2–6 1/2; lip edge swollen; apertural teeth relatively distinct; luster always dull.

Triodopsis alabamensis (Pilsbry, 1902)
(Figs. 27a, 54a–b; Table 8; Fig. 49)

Studied material

(1) TN: Meigs County (GS-105; FMNH 214791): 3 live adults, 7 tissue samples—dissected #2, 4 (illustrated #4); electrophoresed #1, 2, 3, 4, 6, 7; illustrated shell #A.

Triodopsis vannostrandi (Bland, 1875)
(Figs. 16c, 43e–f; Table 2; Fig. 49)

Studied material

(1) SC: Aiken County (GS-179; FMNH 214880): 12 live adults, 12 tissue samples—dissected #1, 8, 12; electrophoresed #1, 2, 3, 4, 5, 6, 10; illustrated shell #11.

Triodopsis hopetonensis (Shuttleworth, 1852)
(Figs. 15a, 42a–b; Table 2; Fig. 49)

Studied material

(1) NC: Catawba County (GS-33; FMNH uncat.): ? live adults, 12 tissue samples—electrophoresed #2, 4, 6, 9. (2) NC: Columbus County (GS-38; FMNH 214827): ca 25 live adults, 25 tissue samples—dissected #15, 25, A (illustrated #A); illustrated shell #22. (3) AL: Perry County (GS-57; FMNH 214832): ca 10 live adults, 13 tissue samples—electrophoresed #8.

Species group *Triodopsis juxtidens*
(Figs. 14c–d, 18a, c, 41, 45a–b, e–f; Table 2; Fig. 49)

Key characters

Penis: penis length moderate; pilaster as in the *rugosa, cragini, tridentata,* and *fallax* groups; wall-pustular columns as in the *cragini* and *tridentata* groups; pore ventrally subterminal, ca 2/5-way from the apex; everted penis shaped as in the *tridentata* group, but with a broader apical knob; large-sized peduncle beneath pore.

Shell: diameter 10–18 mm, moderately to very depressed, whorls 4 1/2–6; umbilicus moderately to very wide; aperture dished but not as deeply as in the *vulgata* group; parietal tooth as in the *vulgata* and *rugosa* groups and the *tridentata* group; palatal tooth marginal to moderately recessed, narrow to moderately broad, squared to pointed; basal tooth marginal, as in the *tridentata* group, but rarely buttressed on the columellar side.

Species subgroup *Triodopsis juxtidens*
(Figs. 14c–d, 18a, c, 41a–d; Table 2; Fig. 49)

Key characters

Shell: palatal tooth rounded, umbilicus moderately depressed to very depressed.

Triodopsis juxtidens (Pilsbry, 1894)
(Figs. 14c, 41a–b; Table 2; Fig. 49)

Studied material

(1) NC: Catawba County (GS-33; FMNH 214838): ca 9 live adults, 12 tissue samples—dissected #1, 2, 3; electrophoresed #2, 4, 6, 9. (2) NC: Burke County (GS-34; FMNH 214839): 1 live adult, 2 tissue samples—dissected #4. (3) NC: Columbus County (GS-37; FMNH 214840): ca 30 live adults, 30 tissue samples—electrophoresed #4, 18. (4) WV: Pendleton County (GS-132; FMNH 214841): 10 live adults, 10 tissue samples—dissected #5, 10 (illustrated #5); illustrated shell #7. (5) WV: Pocahontas County (GS-135; FMNH 214842): 10 live adults, 11 tissue samples—dissected #5, 6; electrophoresed #1, 2, 3, 8, 9.

Triodopsis discoidea (Pilsbry, 1904)
(Figs. 14d, 41c–d; Fig. 49)

Studied material

(1) IL: Hardin County (SC-217; FMNH 214811): 1 live adult, 8 tissue samples—dissected #5 (illustrated #5); illustrated shell #A.

Species subgroup *Triodopsis neglecta*
(Figs. 18a, c, 45a–b, e–f; Table 2; Fig. 49)

Key Characters

Shell: palatal tooth squared; unmbilicus moderately to very wide; depressed.

Triodopsis neglecta (Pilsbry, 1899)
(Figs. 18a, 45a–b; Table 2; Fig. 49)

Studied material

(1) MO: Barry County (GS-96; FMNH 214850): ca 7 live adults, 10 tissue samples—dissected #2, 5 (illustrated #2); electrophoresed #1, 2, 4, 5, 8; illustrated shell #A.

Triodopsis pendula Hubricht, 1952
(Figs. 18c, 45e–f; Table 2; Fig. 49)

Studied material

(1) NC: Wilkes County (GS-149; FMNH 214859): ca 5 live adults, 19 tissue samples—dissected #18 (illustrated #8); electrophoresed #1, 4, 7, 18; measured shell #14.

APPENDIX D. ON THE PHYLOGENY OF THE *TRIODOPSIS FALLAX* GROUP

The *fallax* subgroup is believed to comprise 8 species (Hubricht, 1985). Hubricht (1953, 1971) discussed field evidence for hybridization or lack of it among 6 of these species. In 1975, Grimm cursorily summarized his 10 years of field and laboratory studies on hybridization or lack of it among 7 of these species, and proposed an evolutionary hypothesis based on shell lip and dentition, presence or absence of field hybridization, and current geographical distributions. In Fig. 51, Grimm's (1975) verbal hypothesis is summarized in the form of a cladogram. Table 13 summarizes Grimm's hybridizational evidence in support of this cladogram, and adds the available genetic-distance data. One species is included *(palustris)* which Grimm omitted from his hypothesis.

It is beyond the scope of this paper to evaluate Grimm's (1975) conclusions concerning field hybridization. Grimm's specimens and notebooks are at the National Museum of Natural Sciences, Ottawa, Canada, and deserve morphometric study. The consistency of Grimm's conclusions concerning hybridization with this cladogram is apparent in Table 13: of the 10 species pairs found sympatric, those which commonly hybridize in nature have an average patristic distance (number of transformations separating them) of 2.1 ($n = 6$), those which rarely hybridize in nature have a patristic distance of 3 ($n = 1$), and those which never hybridize in nature have an average patristic distance of 4.3 ($n = 3$). It would be tautological to consider this correlation as validating Grimm's cladistic hypothesis (Fig. 51), however, because he based his hypothesis on these same hybridization data.

An independent test of the cladogram is afforded, however, by the electrophoretic data available for four of the species pairs (Table 13). The four Prevosti genetic dis-

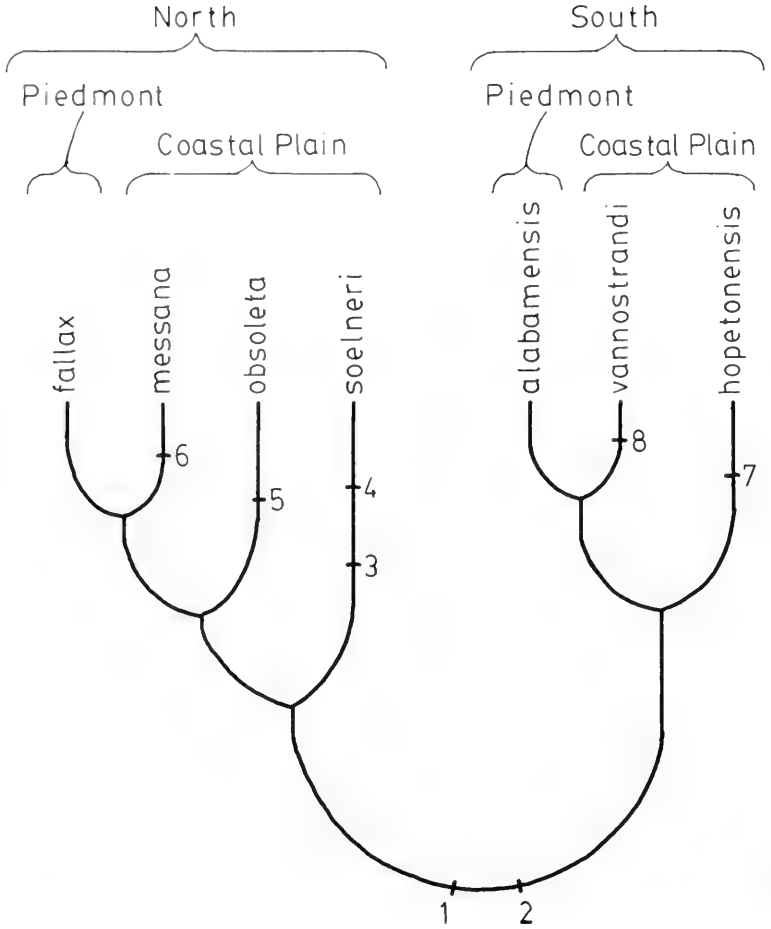

FIG. 51. Cladogram summarizing Grimm's (1975) phylogenetic hypothesis for the *Triodopsis fallax* subgroup. Hypothesized character transformations: 1. Differentiation of apertural tooth prominence into less distinct (Fig. 44a)—to the left—vs. more distinct (Fig. 43a)—to the right—with the ancestral condition unknown. 2. Differentiation of the thickness of the internal edge of the apertural lip and teeth into relatively thin—to the left—vs. relatively thick—to the right—, with the ancestral condition unknown. 3. Type A, extreme reduction of the lip teeth (from Fig. 44a to Fig. 44c). 4. Reduction of overall penial sculpture (from Fig. 17a to 17b). 5. Type B, pronounced reduction of lip teeth (from Fig. 44a to Fig. 42e). 6. Type C, moderate reduction of lip teeth (from Fig. 44a to Fig. 43c). 7. Type D, moderate reduction of lip teeth (from Fig. 43a to Fig. 42a). 8. Type E, slight reduction of lip teeth (from Fig. 43a to Fig. 43e).

tances (taken from Emberton, 1986, Appendix B-1) do not support the cladogram, as is shown below:

Cladistic distance	Genetic distance(s)
2	.32
3	.27
4	.27, .35

where "cladistic distance" equals the number of transformations separating the species in the cladogram (Fig. 51). These data are scant, and the differences not very great, so they are hardly conclusive. Because of the small and close genetic distances involved, this group has probably radiated so recently that electrophoresis will be of little value in deducing its phylogeny; of the currently available biochemical methods, mitrochondrial DNA studies are more likely to provide an-

TABLE 13. Supporting evidence for Grimm's (1975) implied cladogram of the *Triodopsis fallax* group.

Species pair	Patristic distance[a]	Genetic distance (Prevosti)	Grimm's field observations
fallax & *obsoleta*	1	—	hybrids
fallax & *alabamensis*	2	—	hybrids
fallax & *vannostrandi*	3	—	hybrids[b]
fallax & *hopetonensis*	3	—	hybrids
messana & *obsoleta*	2	—	hybrids
messana & *soelneri*	3	—	sympaters[c]
messana & *alabamensis*	3	.27	(no overlap)
messana & *vannostrandi*	4	.27	(no overlap)
messana & *hopetonensis*	4	.35	sympaters
hopetonensis & *vannostrandi*	2	.32	hybrids
hopetonensis & *obsoleta*	4	—	sympaters
hopetonensis & *soelneri*	5	—	sympaters
palustris & *messana*	?	.21	—
palustris & *alabamensis*	?	.22	—
palustris & *vannostrandi*	?	.30	—
palustris & *hopetonensis*	?	.33	—

[a]Number of transformations on Grimm's cladogram (Fig. 51).
[b]"*fallax* × *vannostrandi* × *hopetonensis*."
[c]Although a single hybrid population was found.

swers because of the faster evolutionary rate of this molecule.

According to Grimm's (1975) hypothesis, the *fallax* subgroup consists of a Piedmont stock which has successively invaded and speciated in the Coastal Plain during Plio-Pleistocene regressions, as first suggested by Hubricht (1953). The inland stock differentiated early between *fallax* in the north and *alabamensis* in the south, both with strongly developed apertural dentition. According to the hypothesis, *fallax* spun off three successive Coastal Plain species—*soelneri, obsoleta,* and *messana*—each with a different type of reduced dentition, and ranging from extremely reduced (Fig. 44c), to very reduced (Fig. 42e), to moderately reduced (Fig. 43c); and *alabamensis* spun off two successive Coastal Plain species—*hopetonensis* and *vannostrandi*—each with a different type of reduced dentition, and ranging from moderately reduced (Fig. 42a) to slightly reduced (Fig. 43e). Grimm suggested—for no clearly stated reason—that the longer a species of the *fallax* group remains on the Coastal Plain, the more reduced its apertural dentition becomes. Purportedly, all 7 of these species hybridize in the laboratory, but not all hybridize when they come in contact in the field (Grimm, 1975).

Based on the cladogram of Fig. 51, the *fallax* group into the two subgroups (called "herds" by Grimm, 1975) *fallax* and *alabamensis.*

T. palustris, which was not analyzed by Grimm, is tentatively placed in the (northern) *fallax* subgroup, despite its somewhat southern range (Fig. 49)—and despite Hubricht's (1950–1953) placing it with *alabamensis* for that reason—because of its less prominent teeth and relatively thin inner lip (see transformations 1 and 2, Fig. 51), and because it is electrophoretically closer to *messana* (Prevosti distance .21) than to *alabamensis, vannostrandi,* or *hopetonensis* (Prevosti distances .22, .30, and .33). Following Grimm's concept of evolutionary trends, *palustris's* position in the cladogram (Fig. 51) lies between *obsoleta* and *messana,* because the reduction of its lip teeth (Fig. 42c) is intermediate between those two species (Fig. 42e and 43c).

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GENETIC HETEROGENEITY ON DIFFERENT GEOGRAPHIC SCALES IN *NUCELLA LAMELLOSA* (PROSOBRANCHIA, THAIDIDAE)

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ABSTRACT

The magnitude of gene flow, and hence the potential for population differentiation, in marine mollusks is determined largely by the extent of larval and adult dispersal. In this study we measured population differentiation in an intertidal whelk, *Nucella lamellosa,* which has low levels of dispersal between populations because it lacks planktonic larvae and because adults show little tendency to migrate along shore. Using two polymorphic allozymes, *Pep-2* and *Pgm,* as population markers, we found significant allele frequency differences among 12 breeding colonies sampled on a single low tide along a continuous 100 m boulder beach. These frequency differences, up to 0.12 for *Pep-2* and 0.11 for *Pgm,* arise by random drift in aggregations maintained by homing of adults to previous breeding areas. On a scale of between 100 and 1,000 km, we found considerable differentiation among populations located along the Pacific Ocean coast and in Juan de Fuca Strait, Hood Canal, and Puget Sound. Variation among populations along some shorelines was haphazard as expected from genetic drift in small populations and limited gene flow. In other areas, allele frequencies varied clinally over distances of 300 to 600 km. These clines may have arisen by chance from genetic drift and limited gene flow, or may reflect natural selection on *Pep-2* and *Pgm* or on closely linked loci. A gene diversity analysis indicated that 33% of the total gene diversity was due to population subdivision at various geographic scales and that 67% was contained, on average, within populations. This is the greatest amount of population subdivision yet reported for a marine gastropod and supports the postulate that gastropods with limited larval and adult dispersal should have genetically fragmented populations.

Key words: *Nucella,* protein electrophoresis, allozyme variation, population genetics, microgeographic variation, Pacific Northwest.

INTRODUCTION

Marine gastropods exhibit different modes of larval development which potentially influence the extent of gene flow between populations. Electrophoretic studies of species having long-lived planktonic larvae with a large potential for passive dispersal by ocean currents show that gene flow can have an homogenizing effect on populations (Gooch *et al.,* 1972; Berger, 1973) even in the face of strong regional selection (Johnson & Black, 1984). On the other hand, gastropods with gene flow reduced by larval brooding tend to show much greater levels of differentiation among populations (Berger, 1973; Snyder & Gooch, 1973; Janson & Ward, 1984; Janson, 1986). However, there have been few studies of gastropods with other modes of non-planktonic larval development to demonstrate that reduced gene flow produces a greater amount of genetic differentiation among populations.

In this study, we measured genetic differentiation among populations of *Nucella* [*Thais*] *lamellosa* (Gmelin, 1791), an intertidal

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whelk with much-reduced dispersal during both its larval and adult stages (Spight, 1974). Gene flow is presumably limited by direct larval development in benthic egg capsules and by homing of adults to previous breeding colonies. Although there is some adult migration along shore, populations as near as 25 m can expand and contract independently of one another in response to food availability and reproductive success (Spight, 1974). This reduction in gene flow enhances the formation of local races of shell color, banding and shell sculpturing, which at one time formed the basis of subspecific nomenclature (Dall, 1915; Kincaid, 1957). A study of allozyme variation by Campbell (1978), however, showed that these forms belong to a single polymorphic species.

The goal of this study was to measure the amount of genetic differentiation among populations of this whelk on two different geographic scales. We examined allozyme variation among breeding colonies along 100 m of beach and along 1,000 km of shoreline in Juan de Fuca Strait, Hood Canal and Puget Sound, Washington. If gene flow is restricted to the extent suggested by previous studies of its life history and migratory patterns (Spight, 1974), then significant genetic heterogeneity should be apparent among populations or even among subpopulations.

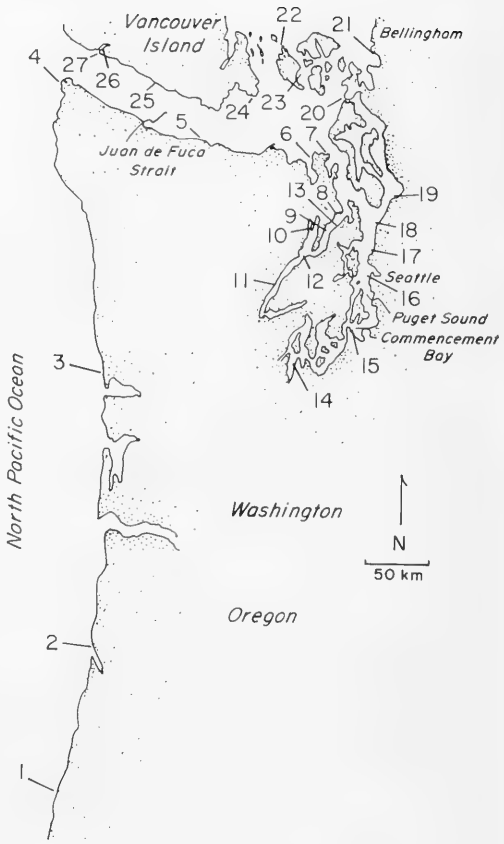

FIG. 1. Locations of samples of *Nucella lamellosa* in the Strait of Juan de Fuca, Hood Canal, and Puget Sound. Location numbers correspond to those in Table 1.

MATERIALS AND METHODS

A total of 2286 whelks were collected from 27 intertidal locations in British Columbia, Canada, and Washington and Oregon, USA (Fig. 1), transported in damp cloth, and kept live in recirculating sea water tanks at 10°C until electrophoresis within one week. Soluble proteins were extracted from foot muscle and digestive gland by maceration in a test tube with distilled water and by centrifugation at 1000 × g for 10 min. Horizontal starch-gel electrophoresis followed May *et al.* (1979) and histochemical stain protocols followed Harris & Hopkinson (1976). Gels consisted of 13% hydrolyzed potato starch (Electrostarch, Madison, WI).

We initially resolved the products of 19 enzyme-coding loci in one sample using three different electrophoretic buffers. A discontinuous system using tris, citric acid, and lithium hydroxide (Ridgway *et al.*, 1970; pH = 8.1) was used for aspartate aminotransferase (*Aat-1, Aat-2;* EC 2.6.1.1), esterase (*Est-1;* EC 3.1.1.1), diaphorase (*Dia;* EC 1.6.2.2), lactate dehydrogenase (*Ldh,* EC 1.1.1.27), peptidase (*Pep-2, Pep-3;* substrate = glycylleucine; EC 3.4.11), phosphoglucomutase (*Pgm;* EC 2.7.5.1), sorbitol dehydrogenase (*Sdh;* EC 1.1.1.14), superoxide dismutase (*Sod;* EC 1.15.1.1). A continuous buffer system using tris, citric acid and N(3-aminopropyl)-morpholine (Clayton & Tretiak, 1972; pH = 6.5) was used for adenylate kinase (*Ak;* EC 2.7.4.3), isocitrate dehydrogenase (*Idh-1, Idh-2;* EC 1.1.1.42), glycerol-3-phosphate dehydrogenase (*Gpd;* EC 1.1.1.8), leucine aminopeptidase (*Lap-1;* EC 3.4.1.1), malate dehydrogenase (*Mdh;* EC 1.1.1.37), and 6-phosphogluconate dehydrogenase (*Pgd;* EC 1.1.1.44). A buffer containing tris, boric acid, and NaEDTA (Markert & Faulhaber, 1965; pH = 8.7) was used for mannosephosphate isomerase (*Mpi;*

TABLE 1. Allele frequencies, Wright's inbreeding coefficient (F) in breeding colonies of *N. lamellosa* at site 21 near Bellingham, Washington.

Number of whelks					
In colony	Electrophoresed	$Pep\text{-}2^{100}$	F_i	Pgm^{100}	F_i
83	50	0.100	0.111	0.890	−0.124
294	60	0.067	−0.066	0.950	−0.053
33	33	0.091	0.267	0.985	−0.026
275	47	0.032	−0.030	0.989	0.022
184	59	0.051	0.300	0.975	0225
124	53	0.009	−0.058	0.962	−0.032
17	17	0.118	0.435	0.912	0.634
52	52	0.038	−0.315	0.923	0.188
21	21	0.095	0.446	0.952	−0.042
63	63	0.127	0.284	1.000	0.0
89	64	0.078	0.348	0.906	0.086
197	72	0.083	0.453	0.931	−0.081
Mean 119.3	49.3	0.071	0.230[1]	0.948	0.040

[1]$P < 0.01$

EC 5.3.1.8), and xanthine oxidase (*Xo;* EC 1.2.3.2). The gel banding patterns for *Pep-2* and *Pgm,* which we scored in all of the samples, were generally well resolved in our samples. Individuals with questionable genotypes were reanalyzed. Representative genotypes of polymorphic loci in each sample were electrophoresed on the same gel to determine allelic identities.

RESULTS

Genetic variation

We examined a total of 19 loci in a sample of 50 whelks and found that *Pep-2* and *Pgm* were polymorphic. We subsequently scored these loci in all samples. There were three zones of banding for gels stained with peptidase using glycyl-leucine as a substrate. The second anodal zone, encoded by *Pep-2,* showed three-banded and one-banded phenotypes reflecting heterozygotes and homozygotes of a dimeric enzyme. We observed a single zone of banding for *Pgm,* which had two-banded heterozygotes and single-banded homozygotes typical of a polymorphic monomer.

Microgeographic variation

We collected samples of whelks from 12 breeding colonies on a 100 m stretch of cobble beach near Bellingham, Washington (site 21) to measure microgeographic variation. We censused all of the breeding colonies that could be found during a single nocturnal spring low tide (8 February 1977) and collected subsamples, or the entire aggregation if it were small (Table 1). Very few solitary whelks were observed away from the breeding colonies. Numbers of whelks in the colonies varied from 17 to 294 and averaged 119.3 (SD = 96.5). This is similar to the average of 145.8 whelks per breeding colony measured by Spight (1974) on San Juan Island. The average nearest-colony distance was 10.1 m which is also similar to the results of Spight (1974) who found intercolony distances of 10 to 15 m.

We had expected intermediate allozyme frequencies for *Pgm* and *Pep-2* at this site based on our results from other localities. However, frequencies for Pgm^{100} in 12 samples ranged from 0.890 to 1.00 with a weighted mean of 0.948, and frequencies for $Pep\text{-}2^{100}$ ranged from 0.009 to 0.127 with a mean of 0.071 (Table 1). Nonetheless, these data may provide some insight into breeding colony structure.

We examined genotypic distributions in the colonies with Wright's (1943) fixation index, F_i, which measures the effects of inbreeding, selection or other processes affecting genotypic frequency. The G-test for goodness of fit (Sokal & Rohlf, 1981) was used to test for the significance of F_i (i.e., departures of genotypic

TABLE 2. Wright's unweighted *F* statistics for *N. lamellosa* on two geographic scales. F_{ST} = gene differentiation among subpopulations relative to the total population. F_{IS} = probability of identity of two homologous genes in an individual relative to its subpopulation. F_{IT} = probability of identity of two genes in an individual relative to the total population.

	Locus	Allele	F_{ST}	F_{IS}	F_{IT}
Site 21 (12 colonies over 100 m)					
	Pep-2	100	0.017	0.252	0.265
	Pgm	100	0.024	0.093	0.115
Average			0.021	0.135	0.190
Pacific Northwest (30 samples over 1000 km)					
	Pep-2	100	0.400	0.116	0.470
	Pgm	100	0.172	0.056	0.218
Average			0.286	0.086	0.344

proportions from Hardy-Weinberg expectations in a sample). F_{IS} is the unweighted average of F_i over samples and is the correlation of two homologous genes in an individual relative to the colony (Table 2). F_{ST} is the correlation between randomly-chosen pairs of homologous genes in a colony relative to the total population. A significant departure from Hardy-Weinberg expectations ($P < 0.01$) appeared for the genotypes of *Pep-2* pooled over colonies but not for *Pgm.* There were no significant departures from Hardy-Weinberg expectations in the colonies themselves, but F_{IS} was 0.252 for *Pep-2* and 0.093 for *Pgm.* The amount of differentiation among colonies measured by F_{ST} was 0.017 for *Pep-2* and 0.024 for *Pgm. G*-tests for independence (Sokal & Rohlf, 1981) of absolute allele frequencies among colonies were significant for both *Pep-2* (G_{11} = 23.1, $0.05 > P > 0.01$) and *Pgm* (G_{11} = 23.8, $0.05 > P > 0.01$).

Geographic Variation

Relative allozyme frequencies and approximate 95% confidence intervals for the most common alleles of *Pep-2* and *Pgm* are arranged along the shoreline in Fig. 2. Frequencies of *Pep-2*^{100} varied clinally from 0.96 at Newport, Oregon (site 1) on the outer coast to about 0.40 at Port Townsend (site 7) (Table 3). The direction of this cline in Hood Canal (sites 8–13), however, was reversed so that frequencies exceeded 0.90 in the southern part of the inlet. In Puget Sound (sites 14–19) the frequencies of *Pep-2*^{100} were less than 0.22 and averaged 0.10. A cline was apparent along the San Juan Islands and the north shore of Juan de Fuca Strait; *Pep-2*^{100} varied from 0.07 at Bellingham, Washington (site 21) to 0.96 at Port Renfrew Harbor, Canada (site 26).

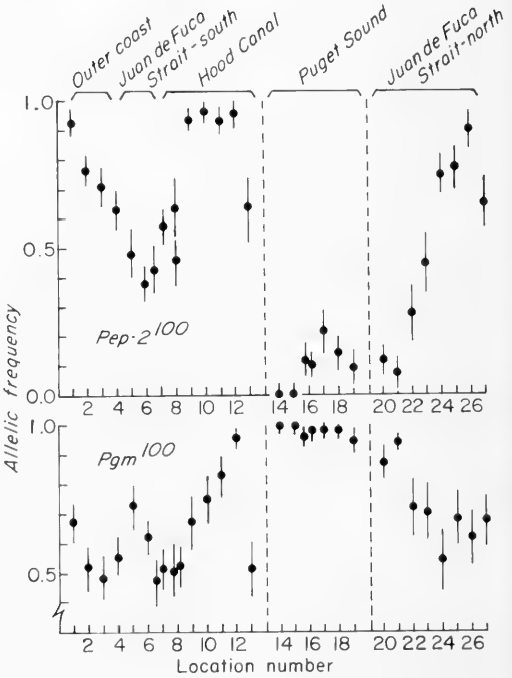

FIG. 2. Allele frequencies of *Pep-2*^{100} and *Pgm*^{100} in populations of *Nucella lamellosa.* Vertical bars represent four binomial standard errors, $[p(1-p)/2n]^{1/2}$, where p is the frequency of the most common allele and n is sample size, and approximate a 95% confidence interval. Broken vertical lines separate geographically isolated groups of samples.

Along the outer coast and Juan de Fuca Strait south, frequencies of *Pgm*^{100} varied haphazardly from 0.48 at site 3 to 0.738 at site 5. In Hood Canal frequencies appeared to

TABLE 3. Locations, dates, number of whelks sampled, allelic frequencies for two polymorphic loci and fixation indices (F) for samples of *Nucella lamellosa* in the Pacific Northwest.

Location	Date (mo-yr)	N	*Pep-2*		*Pgm*				
			100	F_1	100	106	90	109	F_1
Oregon									
1. Newport	8–75	88	0.920	0.38**	0.670	0.330	—	—	0.33**
2. Tillamook	8–75	75	0.760	0.09	0.520	0.480	—	—	0.07
Washington									
3. Westport	8–75	90	0.710	0.38**	0.480	0.520	—	—	0.11
4. Mukkaw Bay	7–75	90	0.620	0.13	0.560	0.440	—	—	0.10
5. Salt Creek	7–75	65	0.474	0.07	0.738	0.261	—	—	0.40*
6. Middle Point	8–75	80	0.394	0.24*	0.630	0.370	—	—	0.12
7. Port Townsend	7–75	57	0.425	0.28*	0.465	0.535	—	—	0.33*
	3–78	80	0.440	−0.01	0.513	0.487	—	—	0.10
8. Hood Canal Bridge	8–75	50	0.640	0.05	0.510	0.490	—	—	−0.16
	3–78	80	0.453	0.02	0.516	0.484	—	—	0.03
9. Big Beef Bay	9–75	50	0.940	−0.06	0.680	0.320	—	—	−0.01
10. Dabob Bay	9–75	50	0.910	0.15	0.750	0.250	—	—	−0.01
11. Hoodsport	11–75	50	0.930	0.53**	0.810	0.100	0.090	—	0.02
12. Scenic State Park	8–75	50	0.960	−0.04	0.960	0.040	—	—	−0.04
13. Kitsap Mem. Park	8–75	50	0.640	−0.04	0.530	0.470	—	—	−0.00
14. Olympia	4–75	40	—	0.00	1.000	—	—	—	0.00
15. Ruston	4–75	40	—	0.00	1.000	—	—	—	0.00
16. Alki	4–75	50	0.120	0.24	0.960	0.040	—	—	−0.04
	8–78	90	0.111	0.32**	0.994	0.006	—	—	0.07
17. Golden Gardens	4–75	50	0.220	0.10	0.970	0.030	—	—	−0.03
18. Edmonds	4–75	50	0.140	−0.16	0.970	0.030	—	—	−0.03
19. Mukilteo	4–75	50	0.090	0.15	0.940	0.060	—	—	−0.11
20. Rosario Beach	7–78	80	0.112	0.25	0.875	0.119	—	0.006	−0.09
21. Bellingham	2–77	591	0.071	0.24**	0.948	0.052	—	—	0.07
22. Snug Harbor	4–75	40	0.450	−0.01	0.700	0.300	—	—	−0.07
23. Friday Harbor	4–75	40	0.275	0.25	0.725	0.275	—	—	0.25
British Columbia									
24. Victoria	5–75	40	0.750	−0.07	0.538	0.462	—	—	−0.06
25. River Jordan	5–75	40	0.763	−0.18	0.688	0.312	—	—	0.13
26. Port Renfrew Harbour	5–75	40	0.900	−0.11	0.612	0.388	—	—	−0.11
27. Port Renfrew Outer coast	5–75	40	0.650	0.34*	0.675	0.325	—	—	−0.25

[1]Wright's (1943) inbreeding coefficient.
*Significant departure from Hardy-Weinberg proportions $0.05 > P > 0.01$.
**$P < 0.01$.

vary clinally from 0.51 at the north-shore entrance (site 8) to 0.96 at site 12 on the south shore. In Puget Sound frequencies varied over a small ranged between 0.94 and 1.0 (sites 14–19). Along the north shore of Juan de Fuca Strait and the San Juan Islands, Pgm^{100} varied irregularly from 0.54 to 0.73 (sites 20–27).

Samples from three sites (7, 8 and 16) were analyzed in 1975 and again in 1978 to measure the temporal stability of allele frequencies (Table 3). Allele frequencies for *Pep-2* varied significantly ($P < 0.01$) between years at site 8 and for *Pgm* at site 25 ($0.05 > P > 0.01$). The remaining 4 comparisons between years were not significant.

F-statistics were originally developed for the analysis of subpopulations within a single population and not for the analysis of populations within a species (Wright, 1943). Nonetheless, we applied this analysis to our allele frequency data for populations on a larger geographic scale knowing that our samples potentially included individuals from more than one subpopulation and making each population a 'subpopulation'. F_{IS} and F_{ST} are as before and F_{IT} is the correlation between homologous genes in an individual relative to the total population that we sampled. For

TABLE 4. Hierarchical analysis of gene diversity for *Nucella lamellosa*. Samples were subdivided into 5 groups for regional comparisons: group 1 = locations 1–4; group 2 = locations 5–7; group 3 = locations 8–13; group 4 = locations 14–19; group 5 = locations 21–27. H_T = total heterozygosity. H_S = mean population heterozygosity averaged over all populations. G_{CS} = relative gene differentiation among colonies within populations. G_{YRS} = relative gene differentiation between years. G_{SR} = relative differentiation among populations within regions. G_{RT} = relataive differentiation among regions.

Locus	H_T	H_S	G_{CS}	G_{YRS}	G_{SR}	G_{RT}
Pep-2	0.472	0.256	0.001	0.002	0.190	0.265
Pgm	0.334	0.264	0.002	0.000	0.092	0.113
Average	0.042[1]	0.027[1]	0.002	0.001	0.141	0.189

[1]Average includes 17 additional monomorphic loci.

Pep-2, 19 of 30 samples had heterozygote deficits, 8 of which represented significant departures from Hardy-Weinberg expectations (Table 3). There was an average deficit of heterozygotes in the samples (F_{IS} = 0.116) (Table 2). For *Pgm,* 14 samples had heterozygote deficits, of which 3 represented significant departures from Hardy-Weinberg expectations. F_{IS} for this locus was 0.056. F_{IT} was 0.470 for *Pep-2,* 0.218 for *Pgm,* and averaged 0.344. F_{ST} values for the 27 locations were 0.400 for *Pep-2* and 0.172 for *Pgm* and averaged 0.286.

We further examined differences among samples with Nei's (1973) gene diversity statistics using the hierarchical algorithm of Chakraborty *et al.* (1982) (Table 4). In this analysis, total gene diversity, H_T, (heterozygosity of pooled allele frequencies) was partitioned into its components which were due to differences (1) among breeding colonies within a location at the lowest level, (2) between years (roughly one generation), (3) among samples within regions, and (4) among regions at the highest level. For regional comparisons the samples were divided into 5 groups corresponding to the outer coast (sites 1–4), Juan de Fuca Strait-south (sites 5–7), Hood Canal (sites 8–13), Puget Sound (sites 14–19), and Juan de Fuca Strait-north including the San Juan Islands and Bellingham (sites 20–27). Assuming that the remaining 17 loci were monomorphic in all samples, H_T was 0.042 of which 0.02% was due to gene differences among breeding colonies at a single location, 0.01% was due to differences between years (measured at three sites), 14.1% was due to differences between localities within regions and 18.9% was due to differences between the five regions. Average heterozygosity per sample varied between 0.0 and 0.053, averaged 0.027, and represented 66.7% of the total gene diversity.

DISCUSSION

Differentiation among breeding colonies

Mature *N. lamellosa* typically aggregate in late winter into small groups in low-intertidal areas in which each female deposits 40 to 60 egg capsules in a common egg mass attached to rocks. Over a five year period, Spight (1974) measured all of the egg capsule masses along a 600 m rocky shore at Shady Cove on San Juan Island and found an average of 50.8 masses each year having an average area of 316.5 cm^2. These egg masses were attended on average by 145.8 whelks. The populations at this site, however, were not necessarily typical of whelk populations at other sites. At a more wave exposed site on San Juan Island, for instance, egg capsule masses were much larger averaging 1586 cm^2 in size and were produced by a correspondingly larger number of breeders.

In our study, site 21 differed from Shady Cove in that it consisted of large cobbles and boulders instead of bedrock. The densities of whelks and of egg capsule masses, however, were similar to those observed at Shady Cove; we found 12 breeding colonies along a 100 m beach that were attended by an average of 119.3 whelks. We therefore feel justified in comparing the Shady Cove data with our own in the following analyses of microgeographic variation.

Our examination of allele frequencies of *Pep-2* and *Pgm* at site 21 revealed a significant amount of allele frequency heterogeneity

among the 12 breeding aggregations. The relative measure of differentiation combined over loci, $F_{ST} = 0.021$, was large considering the physical proximity of the breeding groups. This estimate, however, may be inflated somewhat by sampling errors since our sample sizes were not large. Nonetheless, these results confirm Spight's (1974) conclusion that breeding colonies are not random aggregations of snails along the beach, but are structured to some degree by juvenile site fidelity and by homing of whelks to previous breeding areas.

Island model of migration

Using extensive tagging data and direct observation, Spight (1974) summarized the complex demography of *N. lamellosa* as follows: about 94% of surviving hatchlings stay in their population for their first year and 71% of these remain until they reach maturity three years later, so that 67% of the surviving juveniles spawn for the first time in their own breeding group. Approximately 40% of these spawn a second time of which 71% remain in their original breeding group. Therefore, the probability of a hatchling remaining with the same spawning group is 0.59. This suggests that the migration rate among local colonies may be as high as 0.41. If we assume that breeding colonies are at equilibrium with respect to migration, we can compare this estimate of migration with that predicted by our estimate of F_{ST} and Wright's (1951) island model of migration. Ignoring mutation, relative differentiation among colonies for small migration rates is approximately

$$F_{ST} \approx 1/(4Nm + 1).$$

The model predicts 12 migrants *(Nm)* between colonies per generation over this stretch of beach. Taking Spight's (1974) estimate of average colony size of 146 and our own of 119 whelks as estimates of *N,* the effective migration rate *(m)* is on the order of 0.08 and 0.10, respectively, rather than 0.41.

Both genetic and empirical estimates of migration, however, are subject to several sources of error. First, our small sample sizes would tend to inflate F_{ST} so that the real value of *m* may be larger than our estimate. Second, the island model of migration does not entirely reflect the real biology of *Nucella* in that equal exchange between all colonies is not likely. Spight (1974) has shown that migration among breeding areas varies greatly by area and over time. This again would have the effect of underestimating *m* from the model. Third, the value of *N* used in the model is the effective population size which is undoubtedly overestimated by the census number of whelks in a colony. Longterm sperm storage by females, mating between only a few whelks in a colony, and the presence of immature, parasitized or senescent whelks would inflate estimates of effective population size by direct count. The large positive value of the average fixation index within colonies ($F_{IS} = 0.135$) suggests that individuals in a colony originate from only a few matings. Together with the genetic data, an overestimate of *N* would produce migration rates that were too large. Fourth, the empirical estimate of emigration into other colonies is probably too large because of the mortality of tagged whelks or because of the emigration of whelks out of the study area.

Genetic drift

Microgeographic differentiation has also been reported for the high rocky intertidal, ovoviviparous periwinkle, *Littorina saxatilis* which also has limited adult dispersal (Jansen & Ward, 1984). An $F_{ST} = 0.095$, averaged over 11 polymorphic loci, was found among 11 populations situated along a 1 km beach. Populations as close as 4 m from one another exhibited significantly different allele frequencies. A moderate amount of heterozygote deficit was also observed in these populations ($F_{IS} = 0.070$) and was interpreted to result from partial isolation among populations. Although selection for shell shape between wave-exposed and sheltered sites was strong, allozyme differentiation did not appear to be related to environmental gradients.

Similar small scale differentiation has been reported for other intertidal organisms with differing amounts of gene flow between areas and has been variously interpreted to reflect habitat selection by genotype (Giesel, 1970; Jansen, 1982), genotype dependent spawning times and synchronous larval recruitment (Gosling and Wilkens, 1985), environmental selection among pre-recruit (Johnson & Black, 1984) or post-recruit larvae (Boyer, 1974; Koehn *et al.,* 1976; Gartner-Kepkay *et al.,* 1983; Kartavtsev & Zaslavskaya, 1983), and mixing of recruits from genetically different source populations (Tracey, Bellet & Gravem, 1975; Koehn *et al.,* 1976; Milkman & Koehn, 1977; Lassen & Turano, 1978).

None of these models, however, appears to

explain the pattern of allozyme differentiation we observed among the breeding colonies of *Nucella* on a scale of 100 m. The colonies were located along a gently-sloping cobble beach with an even topography without any obvious longshore gradients in wave-exposure, food availability, or desiccation that could act as selective agents to produce the genetic differences. The formation of breeding colonies in *Nucella* is behavioral, and does not reflect habitat selection during periods of non-breeding when *Nucella* are dispersed along the beach (Spight, 1974). We conclude, therefore, that the allozyme heterogeneity is due to random genetic drift among the small breeding colonies which are partially isolated from one another by homing to previous breeding areas. Additional experiments are required to determine whether whelks converge on microhabitats that enhance larval survival in the benthic egg capsules or whether whelks are attracted to one another by genotype (assortative mating). If assortative mating is important, the analyses of juveniles from a single egg capsule mass may show evidence of inbreeding.

The significant degree of microgeographic variation that we found among breeding colonies of *Nucella* calls attention to our sampling design for studying genetic variation on a larger geographic scale. Most of our samples were taken during nonbreeding times of the year when whelks were dispersed from breeding aggregations. Most of these samples probably included individuals from more than one colony. Thus, the larger number of heterozygote deficits in our samples may reflect the Wahlund effect in which genetically differentiated populations are included in a single sample. This method of sampling, however, tends to average out microgeographic differences in allele frequency and may yield a more representative genetic profile for a region of shore than sampling individual colonies.

Allele frequencies did not vary much over time at the three sites which were resampled after three years. In the later samples smaller, younger whelks were collected to avoid sampling the same adult population twice. Given the degree of site fidelity in this whelk and overlapping generations, temporal changes in allele frequencies would not be expected over such a short period of time. Thus, the two significant differences between years probably resulted from sampling different colonies having different allele frequencies. Changes over longer periods of time, however, might be expected to appear through genetic drift.

Regional differentiation

We observed a marked difference in the levels genetic diversity within and among samples from Hood Canal and those from Puget Sound. In Hood Canal, allele frequencies varied widely over a range of about 0.50 for both *Pep-2* and *Pgm,* whereas in Puget Sound allele frequencies varied over a much narrower range of about 0.20. In the lower most reaches of Puget Sound to the south of Commencement Bay, populations of *N. lamellosa* are scarce and the total lack of heterozygosity at sites 14 and 15 is most likely due to the loss of alleles through drift in small populations. This whelk is much more abundant at other locations in Puget Sound, however, and drift in small population is an unlikely explanation for low levels of genetic diversity.

Another explanation may be that Puget Sound is polluted by industrial waste to a greater extent than Hood Canal and pollutants may be acting as selective agents on *Pep-2* and *Pgm* or on linked loci. For example, a smelter is located in Commencement Bay that historically released heavy metals into marine waters (Bromenshenk *et al.,* 1985). Complexed metals such as mercurial oxides have been implicated as selective agents on allozymes in some marine organisms (Nevo *et al.,* 1984). In addition, wood processing plants release sulfide compounds and accidental spills of oil and other toxic substances are not uncommon.

In some areas, notably among sites 1–7 and 20–27 for *Pgm,* allele frequencies varied haphazardly among populations as expected if random genetic drift and restricted gene flow were the most important influences on allele frequencies. In other areas, principally along the Strait of Juan de Fuca for *Pep-2* and among locations in Hood Canal for *Pgm,* allele frequencies varied clinally. One explanation is that these clines reflect contact between differentiated populations that invaded uninhabited shores after Pleistocene glaciers receded from the Pacific Northwest after a glacial maximum about 18,000 years ago (Esterbrook, 1969). Kincaid (1957) suggested that repeated post glacial invasions may explain the geographic distributions of the various shell morphs of *N. lamellosa.* It is, however, not possible rigorously to test these

historical hypotheses with the present set of genetic data.

Another explanation is that the allele frequency clines appeared by chance (Endler, 1977). Clines resulting from drift and gene flow alone should appear independently of one another for different loci. This appears to be the case for some of the clines that we observed. A sharp cline exists for *Pep-2* among outer coast and south shore Juan de Fuca Strait populations but not for *Pgm* over this same coastline. There are, on the other hand, parallel clines for both loci along the north shore of the Juan de Fuca Strait and into Puget Sound. These clines may simply be coincidental or may be the result of a common selective agent.

Clines may also arise by adaptive differentiation in response to selection along smooth or abrupt environmental gradients (Endler, 1977). Such clines have been reported for a *Lap* locus in the intertidal mussel, *Mytilus edulis* (Hilbish & Koehn, 1985). It is difficult in most cases, however, to determine whether selection is acting on allelic products of a particular locus or on those of linked loci. In addition to numerous physical, chemical and biological oceanographic gradients along the shores of Juan de Fuca Strait, Hood Canal, and Puget Sound, several sources of marine pollution exist in Puget Sound that may act as selective agents. Additional studies are required, however, to assess the importance of selection on allele frequencies.

Gene flow

Although the lack of planktonic larvae suggest that gene flow is limited, other kinds of passive dispersal may still be important for gene flow in *N. lamellosa.* Passive transport can be achieved by the attachment of egg capsules to floating logs or algae, or capsules may be dislodged by storms and carried to other locations by currents (Kincaid, 1957). Palmer (1984b), however, discounted such mechanisms for *N. emarginata,* which deposits intertidal egg capsules similar to *N. lamellosa,* because drifting capsules would most likely be captured by anenomes, drift into the strand line and die or settle into subtidal areas with little chance of survival. Our demonstration of differences among colonies at a single site and among populations separate by a few kilometers (e.g. sites 8, 9, 10 and 13), suggests that any form of passive migration is not significant.

Nonetheless, gene flow and migration along a shore must occur at some rate over long periods of time, else how would postglacial shores become inhabited? Even species such as the house mouse *(Mus musculus)* (Baker, 1981) and the land snail *(Partula taeniata)* (Murray & Clark, 1984), which consist of strongly isolated populations, have been shown, through the introduction of genetic markers, to have significant rates of gene flow over time. The existence of populations of *N. lamellosa* that are fixed or nearly fixed for different alleles would facilitate transplant experiments to measure long term rates of gene flow. Such experiments would, of course depend on the successful introgression of the introduced genes into a population.

Geographic range and speciation

Nucella lamellosa appears to have the greatest amount of genetic fragmentation among populations of any gastropods studied so far with electrophoresis (Table 5). Our results for *N. lamellosa* showed that 33% of the total gene diversity was due to subdivision on different geographic scales. Another intertidal gastropod, *Littorina saxatilis,* which is ovoviviparious giving birth to crawlaway juveniles, also shows considerable genetic differentiation among populations. An analysis of gene frequencies combined over three studies (Ward & Warwick, 1980; Janson & Ward, 1984; Janson, 1987) indicated that 11% of the total variation was due to all sources of microgeographic and geographic subdivision. Other gastropods, which have planktonic larvae and as a consequence presumably greater gene flow between populations, exhibit much less genetic fragmentation among populations. Studies of *Nassarius obsoletus* (Gooch *et al.,* 1972), *Crepidula fornicata* (Hoagland, 1984) and *Siphonaria jeanae* (Johnson & Black, 1984a, b) show that populations separated by as much as 2,000 km have not diverged genetically from one another. In these species the amount of variation due to all sources of population subdivision is less than 5% and in some cases less than 1%. The salt marsh pulmonate, *Melampus bidentatus,* while having planktonic larvae, shows a large degree of population fragmentation (Schaeffer *et al.,* 1985). In this case oceanographic barriers limit larval dispersal and gene flow. These studies substantiate the hypothesis that

TABLE 5. Hierarchical gene diversity analysis (Nei, 1973) of geographic and temporal variation in 6 gastropods.

Species	No. of samples	No. of loci	G_{ST}						Reference
			Temporal	1 m to 1 km	1 to 10 km	10 to 100 km	100 to 1000 km	Within sample	
Nonplanktonic larvae									
Nucella lamellosa	41	2	0.000[1]	0.002	—	0.141	0.189	0.668	This paper
Littorina saxatilis	42	5	—	0.023	0.053	0.021	0.021	0.890	Ward & Warwick (1980 Janson & Ward (1984) Janson (1984)
Planktonic larvae									
Crepidula fornicata	7	19	0.007	—	—	0.010	0.043	0.941	Hoagland (1984)
Melampus bidentatus	6	10	—	—	0.113	0.145	—	0.742	Schaeffer et al. (1985)
Nassarius obsoletus	7	2	—	—	—	0.002	0.001	0.997	Gooch et al. (1972)
Siphonaria jeanae[2]	51	4	0.000	0.000	0.000	0.000	0004	0.996	Johnson & Black (1984)

[1]Values greater than 0.0 but less than 0.00001.
[2]Measured with F_{ST}.

planktonic larval dispersal—if it occurs—acts as a strong homogenizing force among populations.

There is considerable interest in the relationship between modes of larval development, its influence on gene flow and population structure on one hand, and geographic range, speciation and extinction on the other. Shuto (1974), Crisp (1978), Sheltema (1978), and Jablonski (1986) postulate, in part, that species with widely dispersing planktonic larvae resist speciation through the cohesive effect of gene flow. Such species also tend to have large geographic ranges because they can easily invade favorable habitats. On the other hand, species with reduced gene flow have much greater genetic fragmentation among populations which is thought to produce a greater rate of speciation. Such populations are also thought to have shorter geographic ranges because colonization is retarded by reduced larval dispersal.

Although the lack of larval and adult dispersal in *N. lamellosa* produces a genetically fragmented population structure, this whelk does not appear to fulfill the predictions of the foregoing hypothesis. Contrary to prediction, *N. lamellosa* has one of the largest geographic ranges of North Pacific Ocean gastropods extending over 30° of latitude from Monterey, California to the Bering Sea and along the Aleutian Archipelago. *Nucella emarginata,* which also has the same mode of larval development, also occupies an equally large geographic range along the Pacific Coast of North America (Palmer, 1984b). It may be that the threshold of gene flow required to bring about species cohesiveness is generally much lower than is assumed in these arguments. From theory, substantial differentiation may be prevented by only a single migrant per generation (Spieth, 1974). Alternatively, *N. lamellosa* as it is presently defined may include more than one taxonomic unit. This appears to be the case for *N. emarginata* where Palmer (1984a) recently discovered a genetically distinct sibling species in California. Clearly, additional genetic studies of *N. lamellosa* are needed to test the predictions of the gene flow-speciation hypothesis more rigorously.

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THE COLOURS OF MARINE BIVALVE SHELLS WITH SPECIAL REFERENCE TO *MACOMA BALTHICA*

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ABSTRACT

Colours of the shells of marine bivalves have been dismissed as mere excretory products. Some internal colours may be present for reasons other than their visible properties. The British species, when scored for the brilliance or weakness of their external colours and for their degree of probable exposure to visual predators, suggest strongly that there is a connection between these variables, probably because of the action of visual predators. Very small shells show no patterning, and are left out of this comparison.

While many species may be cryptically colored, others clearly are not. The shell colour variation in *Macoma balthica* is shown to be a definite polymorphism, not continuous variation, and descriptions of the morphs are given. As all are conspicuous against the normal background of mud or muddy sand, it is probable that the polymorphism is maintained by apostatic selection, except that a sample from the Baltic Sea may be cryptic. Examination of the nature of the variation in this species against predictions from a colour atlas of what would be expected if only apostatic selection were acting, suggests that some other factors must be acting as well as apostatic selection.

Key words: coloration; marine bivalves; *Macoma balthica;* shell; polymorphism.

I. INTRODUCTION

In the general account of bivalves in the *Treatise on Invertebrate Paleontology,* Cox (1969, but probably written long before) remarks "The pigments are thought to be waste products of metabolism, derived from the diet or other sources, and secreted in the shell as a means of disposal. As in the gastropods, it is improbable that the colour ornament can have any protective function in the great majority of bivalves which lie buried in sediment. Some bottom-living forms, notably the pectinids, appear remarkably well camouflaged."

Sufficient work has been done on terrestrial gastropods (Cain, 1983; Heller, 1975) and some on marine ones (Reimchen, 1979) to suggest that this viewpoint is untenable for them, and an excellent paper on the bivalve *Donax faba* (Gmelin) by D. A. S. Smith (1975) hardly encourages its application to bivalves. Moreover, a disposition to regard bivalve shell colours as mere waste products would no more conduce to their proper investigation than does the insistence by Gould & Lewontin (1979) that discordant patterns in bivalves are in origin non-adaptive, and merely the result of constraints. A survey of the colours (and patterns) of bivalve shells in relation to what is known of the ecology and habits of the species (this paper) suggests strongly that when certain irrelevant colourings are put aside, coloration does show regularities like those found, for other animal groups, in Cott's classic work (1940).

While much coloration in bivalves may well be cryptic, there are various species, both richly coloured and variable, that seem to be conspicuous. When the intellectual stumbling-block of *a priori* non-adaptation is removed, it becomes possible to consider whether such species do show adaptation of any sort. In most bivalves that vary in colour, the nature of the variation has not been described accurately. The purpose of the present paper, after surveying the colours and patterns of British bivalves in general, is to examine the variation in the very common species *Macoma balthica.* The variation is found to be a true polymorphism with some peculiar features. Its significance in the life of the animal is discussed.

II. COLOURS OF MARINE BIVALVES

(i) Classification of bivalves

For the purposes of this paper, the classification given in the *Treatise on Invertebrate*

Paleontology is used, except that the superfamily Mesodesmatacea is added (Yonge & Allen, 1985). The difficulties in determining the proper classification of early Palaeozoic bivalves (and indeed whether some of them are bivalves or Crustacea) can be appreciated from the papers of Newell & Boyd, Pojeta, and Scarlato and Starobogatov in the symposium edited by Yonge & Thompson (1978). While the classification of the *Treatise* may not be final, it is most likely to be altered either by reallocation of some of the earliest groups (which do not concern the present paper) or by recognition of convergence, which will merely strengthen the points made in the present discussion.

(ii) Types of colours

From the point of view taken in the present paper, the colours of bivalve shells can be divided into three types.

(a) Iridescences

The famous mother-of-pearl, and real pearls, which together with Tyrian purple caused Pliny to regard molluscs as the root of all evil—see Rackham (1940)—show iridescence. This is the result of an extremely regular submicroscopic packing of the structural elements of the shell, producing (in effect) diffraction gratings. Such iridescence is normally internal, on the insides of the valves, or (in gastropods) hidden under a thick periostracum. There is no need, therefore, to consider them as subject to visual selection by predators (although this possibility cannot be excluded for some trochid gastropods which normally wear to show some iridescence externally).

(b) Structural melanins

It was pointed out to me many years ago by Professor N. Tinbergen that the flight-feathers of gulls show less wear in their black than in their white areas, which is easily verified on moulted remiges picked up on the beach. Dr. Carol Jones tells me (personal communication) that horses' hooves, when of a light golden colour, split and wear much more than the normally-coloured ones. It seems, therefore, either that melanins, and perhaps other dark pigments, confer additional hardness where they are secreted into skeletal structures, or those structures are hardened when specially modified to receive such pigments. Many bivalve shells have dark blotches or flushes of pigment internally, sometimes in the muscle scars, often on the upper edges of the valves on either side of the umbones. Even in shells which are normally plain white inside, such marks may occur as individual variants, as can be seen in the common cockle, *Cerastoderma edule* (L.). There appears to be a range of intensities in melanins varying from dull yellow to brownish orange, brown, black-brown, and black. Some purples, blues, violets and blue-blacks are found in the same positions, and, whether melanins or other genera of pigments, probably have the same effect. Such colours as these in the insides of shells may possibly be related to a strengthening of the shell and, like iridescences, need not be considered either as subject to selection by visual predators or in any sense a disproof that externally visible colours are in any way adaptive. Other internal colours may have a different function.

(c) Externally visible colours

These are the colours that are investigated in this paper. They range from brilliant reds, oranges, yellows, browns and blacks to occasional greens, blues and violets. In many bivalves they are more apparent in young shells than in old ones, young shells often being more translucent, so that pigment distributed in the thickness of the shell contributes more to the external appearance. Normally or occasionally exposed parts of the animal itself often show cryptic, disruptive, or flash colour, for example the ends of the siphons of *Hiatella arctica,* the mantle tentacles and perhaps the whole body of *Lima* when forced to escape by rapid swimming, the brilliant red foot of species of cockle—again when jumping in an escape reaction. I have not been able to collect sufficient data to illustrate this point in detail.

(iii) Types of variation

To my knowledge, no exact description of the variation in colour and pattern of any British bivalve, has yet been published (and very few for any foreign one). Even in the standard monographs, such as Forbes & Hanley's (1853) or Jeffreys's (1863–69), often no more than an indication of the range of

variability is given. In particular, no careful separation of discontinuous from continuous variation based on random samples has been made. Museum samples cannot be taken to be random, and the bestowing of a varietal name is no indication whatever of whether the form concerned segregates or is part of a continuum, as has been shown extensively in the gastropod *Cepaea* (compare Cain, Sheppard & King (1968) and references therein with Taylor (1914)).

I have been forced, therefore, to use a very rough classification of bivalve variation from the data in the standard monographs and Tebble (1966), and from my own experience, which is not extensive. If a species is stated to show several markedly different shell colours, and/or different patterns, or the frequent presence and absence of a pattern, it is classed as I, *highly coloured and variable.* This class certainly contains some true polymorphisms (*Macoma balthica* is an example) but other species in it may show continuous although extensive variation.

If a species is described as very far from white, although with no great range of coloration, e.g. with a black or black-brown periostracum, or a bright brown shell, or, as in *Glycimeris,* with a constant mottling all over of yellow-brown, it is classed as II, *well coloured.* If a species is given as pale-coloured, or occasionally tinged with colour, or with scattered or inconspicuous markings, it is classed as III, *poorly coloured.* If it is described as off-white, dirty white or white, it is classed as IV, *white.* Probably some rather translucent shells included here ought to be transferred elsewhere if well-marked colours of the animal show through; the genus *Lima* is an example. On this grouping, the percentages of British species are I 12.80, II 20.12, III 27.44, and IV 39.63.

The above classification is very rough, and exactly where some species should be is a matter of opinion. To make the allocations clear, appendix A gives a complete list of the British species, following Tebble (1966), with their classification in this paper. I shall be glad to receive corrections accompanied by better data, and if possible random samples.

The British archipelago is fortunately situated for the present purpose, since it is the meeting-place of three faunas, Lusitanian-Mediterranean, north-west European (sometimes called Celtic) and sub-boreal. For its area, it has a large number of species of marine bivalves.

(iv) Sources of data for probable exposure to predators

Tebble (1966) seems concerned to give the full range of occurrence in respect to both depth and types of substrate for each species, a treatment which tends to obscure their stations of greatest frequency and abundance. Much on this subject has been gleaned from Yonge & Thompson (1976) and Yonge's *New Naturalist* volume (1949). In addition, Barrett & Yonge's guide to the sea shore (1958) and the illustrated account of marine molluscs of the English Channel and French Atlantic coasts by Bouchet *et al.* (1979) have been used, as well as a variety of papers that are noted under each species in Appendix A.

(v) Variation in relation to size

If, as seems probable (Cott, 1940), patterning of the shell serves to break it up visually, or to disguise it as part of a patterned background, it might be expected that very small shells, being at or below the commonest blotch size in the environment, would not show patterns. A pattern means here any marked variation in colour over the outside of the shell. The 20 British marine bivalves given by Tebble as 0.5 cm or less in their greatest measurement when full-grown are given in Table 1, with relevant data. This size-limit was chosen somewhat arbitrarily but bearing in mind the breadths of the finer rays on mussels or *Mactra corallina,* and of the mottlings on *Glycymeris glycymeris* and various scallops. None of these small species have any patterning at all reminiscent of the forms just mentioned; indeed, the only trace of variation in colour over the shell is in *Turtonia minuta,* to which *Lasaea rubra* might be added, and the only marked variation between individuals is in *Astarte triangularis* which may have a colour polymorphism. Of these three species, the first two are common intertidally in rock crevices, empty barnacle shells and the like, and are probably often exposed to visual predators. The *Astarte* lives off-shore in sandy mud, sandy gravel and shell gravel (Tebble, 1966) and its exposure to visual predation needs investigation. Of the remaining species, of 0.51 cm and more, 23 have a definite pattern, 37 are recorded as with or without a pattern (perhaps polymorphic?) and 104 as without a pattern (Table 2). When those with a pattern are combined with those

TABLE 1. British marine bivalves less than 0.5 cm maximum dimension; external colours of shell. Nomenclature and arrangement as in Tebble, 1966.

Species	External colour	Size (cm)
Yoldiella lucida	greyish green	0.32
Yoldiella tomlini	greenish or brownish yellow	0.48
Phaseolus pusillus	whitish	0.16
Arca pectunculoides	straw-coloured	0.48
Crenella decussata	yellow-brown	0.32
Crenella prideauxi	pale yellow	0.32
Lima sarsi	cream, translucent	0.32
Astarte triangularis	light yellow, orange or dark brown	0.32
Thyasira croulinensis	white	0.32
Thyasira ferruginea	rusty encrustation	0.32
Thyasira subtrigona	translucent	0.16
Lasaea rubra	light yellow, tinted reddish	0.32
Lepton nitidum	light yellow	0.32
Neolepton sulcatulum	white	0.16
Neolepton sykesi	translucent	less than 0.16
Epilepton clarkiae	pale yellow or white	0.16
Devonia perrieri	white, occasionally tinted brown	0.48
Montacuta substriata	whitish or translucent	0.32
Mysella bidentata	light brown	0.32
Turtonia minuta	brownish with purplish or rose at umbones	0.32

with one sometimes (to obviate low expectancies), (Table 3) a χ^2 with 5 degrees of freedom of 21.39 (P just less than 0.001) results, which is highly significant. A more extended analysis of pattern occurrence against size is given in Table 2, which suggests that 1.0 cm could be taken as the upper limit of small size in future analyses. It also suggests that the proportion of patterned forms is markedly higher for shells above 4 cm than for those from 1 to 4, and this is confirmed on a separate χ^2 for the two classes of size 1.01–4 and 4.01–32; ($\chi^2_1 = 7.65$, $P < 0.01$).

In the rest of this paper, therefore, shells below 0.5 cm are omitted.

(vi) Variation in relation to exposure

By means of the information gleaned from the sources mentioned above, plus some experience of my own of intertidal and other bivalves, a rough classification has been made into (i) those wholly or frequently exposed to view, (ii) those only partially exposed to view, and (iii) those probably entirely concealed. Under (i) are all epifaunal bivalves, such as edible mussels and oysters, and such forms as many scallops which, although they may nestle into sand, remain very superficial, and may be often fully visible, e.g. when swimming. This category merges into the next, and again, the allocation of particular species is a matter of opinion. Under (ii) are forms which are shallow burrowers, and those that lurk in shallow crevices such as occur in the holdfasts of large seaweeds. Some, such as *Nucula,* are so slightly buried that they could be very easily exposed by predators. *Lima hians,* which is a nest-builder, has been placed in group (i) because its well-known escape reaction by rapid swimming, the brilliant colour of its tentacles, and its apparently distastefulness (Gilmour, 1963, 1967) suggest that it is not infrequently disturbed by predators; the other limids have been put in group (ii). In group (iii), again not separated by any clear division, are nestlers in deep crevices, burrowers in thick black muds such as *Thyasira,* permanent deep burrowers unable to burrow again if exposed, such as *Mya,* actual borers, and deep-sea forms. The percentages of British species in these categories are (i) 20.12, (ii) 45.12, and (iii) 34.76.

Stanley (1970) rightly refers to *Donax* as burrowing rapidly and deeply. However, as Ansell (1968, 1983) shows, several species of it feed on the surface of the sand, migrating upwards with the waves breaking on the beach. The rapid burrowing is an escape

TABLE 2. Distribution of British marine bivalves by size and type of variation. Size in cm.

	0–0.5	0.51 to 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Total
With pattern			5	3	1	1	3	2	1	2			1	1			1		1			1											23
With or without pattern		2	9	2	5	1	4	5	1	2		2		1			2		1														37
With no pattern	20	10	34	14	14	4	4	8	4	2		2		5			1							1								1	124
Totals	20	12	48	19	20	6	11	15	6	6		4	1	7			4		2			1		1								1	184

TABLE 3. Distribution of British marine bivalves by size and type of variation. Lumped data.

Size class	0–1.0 cm	1.01–2	2.01–4	4.01–6	6.01–8	8.01–32	Totals
With any pattern	2	14	11	9	9	15	60
With no pattern	30	34	28	8	12	12	124
Totals	32	48	39	17	21	27	184
% with pattern within size class	6.25	29.17	28.21	52.94	42.86	55.55	

χ^2, 5 degrees of freedom = 21.39, $P < 0.001$.

TABLE 4. Distribution of species of British marine bivalves according to probable degree of visibility to predators (categories (i)–(iii)) and degree of coloration and patterning (types I–IV). Shells with usual adult maximum measurement less than 0.5 cm excluded.

	Category (i) well exposed to predators	Category (ii) partially hidden	Category (iii) hidden	Totals
Type I highly coloured and variable	10 (7.89)e	11 (0.25)e	0 (7.30)d	21
II well coloured	12 (4.31)e	20 (1.75)e	1 (9.56)d	33
III poorly coloured	7 (0.47)d	26 (1.60)e	12 (0.85)d	45
IV white	4 (6.30)d	17 (5.18)d	44 (20.29)e	65
Totals	33	74	57	164

In each cell the upper number is the number of species; the number in parentheses is the contribution to the χ^2.
e = excess, d = deficit of observed against expected numbers.
χ^2, 6 degrees of freedom = 65.75, $P << 0.001$.

reaction, but the important point for the present paper is the animal's exposure to predators, both birds and crabs, on the shore. Probably even the British ones, which are less mobile, should be in category (i) but I have preferred to leave the genus in (ii), erring on the cautious side. Cockles are well known to have a jumping reaction, using the muscular foot. Ropes & Merrill (1973) note leaping and gliding in *Spisula solidissima,* (Dillwyn) especially young ones. Ansell (1967) describes leaping in *Gari tellinella* and *G. fervensis,* and provides a review of the whole subject (1969). Many shallow burrowers (and even some razor shells, in spite of their ability to disappear down their burrows at amazing speed; see McMahon & McMahon, 1983) show some leaping movement. Even when it is an escape mechanism from starfish, it may still expose them to other predators.

(vii) Tentative conclusions

The distribution of species by their degree of coloration and probable degree of visibility to predators is given in Table 4. The resulting χ^2 with 6 degrees of freedom is highly significant, and inspection of the contributions from

each cell shows that there are marked excesses over expectation of white shells in category (iii) (concealed), and of highly coloured and variable shells (type I) in category (i). Correspondingly, whites are deficient in (i), and type I and type II shells in (iii). A closer consideration of the species in each cell suggests that a detailed examination of apparent exceptions would be well worth while. Thus in the shallow burrowers, poorly coloured or white shells, e.g. in the cockles and venerids, tend to have strong sculpture, or to resist breakage by their thickness. *Montacuta ferruginosa* (II, iii) is not normally exposed and may owe its rusty coloration to staining in the rusty-coloured anal track of its host, an irregular urchin (Marshall, 1891; Morton J. E., 1962). *Galeomma turtoni* (IV, (i)?) is recorded as crawling about like a gastropod, and one species of the genus gives what appears to be a dymantic (frightening) colour display (Morton, B., 1975). Apparent exceptions, therefore, may be no exceptions in reality, when their habits are fully known.

It appears, then, that overall, there is reason to believe that the superficial colours and patterns of British marine bivalves are highly influenced by selective agents related to degree of exposure; if these include visual predators, type II forms may be expected to be cryptic (e.g. some *Nucula* spp., *Arctica islandica, Glossus humanus*), being seen by predators on dark muds or muddy sands. Type I forms may be cryptic on diversified backgrounds, as Cox (1969) allowed for scallops, or apostatically coloured as Smith (1975) suggested for *Donax*. Type III forms may perhaps be cryptic, especially when young, on paler backgrounds, or more often be normally invisible, and so need no coloration. It seems unlikely that direct selective action by the physical factors of the environment (e.g. insolation, winter temperature etc.) should produce the diversity of colours seen in Type I forms. A first hypothesis, therefore, is that colour variation is principally selected for by visual predators. It will be seen below for *Macoma balthica* that the situation cannot be as simple as this.

Stanley (1970) has paid careful attention to the modes of life of a large number of marine bivalves in New World waters in relation to the shape and other characteristics of the shell. He did not consider colour, since he was primarily concerned with the interpretation of fossil bivalves, in which it is very seldom preserved, and indeed whitened artificially his modern shells before photographing them (in order to bring out the surface sculpture). A comparison of his data with the colours and patterns noted briefly by Abbott (1974) and Warmke & Abbott (1962) for each of his species, suggests the same conclusions as those reached for British bivalves. A fuller treatment is in preparation.

It will be seen from the classification given in Appendix A that type I shells are found in the subclasses Pteriomorpha (Pectinidae) and Heterodonta (order Veneroida, superfamily Tellinacea, families Tellinidae, Donacidae, Psammobiidae; superfamily Veneracea, family Veneridae). A glance at Abbott (1974) confirms these families and possibly adds to the Pteriomorpha the superfamily Limopsacea (family Limopsidae or Philobryidae, *Limopsis antillensis* Dall, with pink, orange or yellow shells). Certainly the family Spondylidae is to be added to the Pectinacea. In the Heterodonta (order Veneroidea) the superfamily Chamacea (e.g. *Chama macerophylla* (Gmelin), "lemon-yellow, reddish brown, deep- to dull-purple, orange, white or a combination of these colours" Abbott (1974) achieves type I status. In the same order but in the superfamily Carditacea, *Pleuromeris tridentata* (Say) appears to be of type I ("grayish brown to bright-rose, sometimes with red-brown mottlings"). The subclasses Palaeotaxodonta, Cryptodonta and Anomalodesmata seem to achieve at best only type III or IV coloration, although a few palaeotaxodonts in the British fauna (*Nucula* spp.) can be ranked as type II.

It is obvious even from this brief survey that type I coloration shows much convergent evolution. It seems most probable that its distribution is determined by mode of life, not by taxonomic affinity.

III. COLOUR VARIATION IN *MACOMA BALTHICA*

(i) Materials and methods

(a) Collections

Samples of freshly dead shells were collected on the strandline, and nearby, at Red Rocks and Hoylake (Wirral Peninsula) after a considerable shell-wreck by A. J. C. and J. T. Cain in late December 1983 and early January 1984. A first sampling was by picking up all shells seen within small areas, to avoid

visual bias. Later samples were scooped up with a sieve from the vast numbers of shells huddled along the strandline and partly buried in the churned sand. Much of the sand was washed away from the sieve in tide-pools, and the rest removed by more leisurely washing at home. No visual bias could have been exerted in these samples; since they agreed well in proportion with earlier samples, these also can be accepted as without collector's bias. Only complete shells, with the valves attached to each other by the ligament were used in our samples, except that a few with one valve partly damaged were not rejected.

A further collection of shells made at Red Rocks, Wirral, between the areas collected by us was kindly given us by Dr. Ian Wallace (Liverpool City Museums) in 1985. This also was scooped up from a strandline, and is free from visual bias. A collection, almost entirely of separate valves, from near Camber Sands (south coast of England) by Dr. J. Mallet was picked up by eye, and Dr. Mallet queries (in litt.) its randomness on the grounds that yellow shells are very noticeable. Interestingly, this sample does show an enhanced proportion of yellows as compared with the rest of those from the Wirral Peninsula but otherwise has much the same colour range as our own collections. Through the kindness of Dr. G. Russell a random collection of specimens (preserved) has been received from the Baltic Sea (Finnish coast; Tvarminne Zoological Station). This differs entirely from the others, as can be shown by a rough score. It was found impractical to remove the animals without considerable risk of breaking the shell. Linnaeus (1758) rightly characterised the Baltic population as *fragilissima.* Voucher specimens are being deposited at the Academy of Natural Sciences of Philadelphia.

(b) The Villalobos atlas and colour scores

To give repeatable colour scores, and to explore the actual in relation to the possible colour variation, the colour atlas by Villalobos and Villalobos (1947) was used. The atlas is an analytical one, with each of the 38 pages generated from a different hue, from red through the spectrum to blue and purple, the series being a continuum. On each page (Fig. 1), the top line of colour squares is the series of neutral tints from darkest (1) to palest (20) and successive lines down to the bottom of the page increase in the intensity of hue and decrease in the content of neutral tint. The first row (0°), the same on each page, is totally unsaturated, showing only neutral colour; the last (12°) is completely saturated with the hue. From left to right, the columns decrease in the intensity of pigment (both of hue and of neutral tint) from 1 to 20, several of the very palest in columns 18 and 19 not being printed, the very pale shades being extremely difficult to print. A few that were printed have been cancelled subsequently. Column 20 comprises only the white square in row 0°.

On each page, therefore, the most intense colour is seen in row 12°, columns 12–16, the numbers lower than these being more intensely pigmented and therefore more sombre, the higher ones being paler. The general muddiness of colour increases up the page and to the left, until in row 0° column 1 we have virtual black, the intensest neutral tint.

The pages can therefore be thought of as half sections of a cylinder, radiating from a central axis, the neutral-tinted row 0° which is the same on every page, with the brightest spectral colours as a band around the periphery of the cylinder and well above its middle. Pure white shells are scored as row 20.

The principal difficulties in the use of the Villalobos atlas for scoring variation in *Macoma balthica* are twofold. Some hues fall between two pages, and while they can be scored to a good approximation, an atlas with twice the number of pages would be desirable. In the following scores, (see Table 5) 2/3 (for example) indicates a hue falling between pages 2 and 3. If it is clearly closer to 3 than 2 it is given as −3, if closer to 2 than 3 it is 2+. Further, and much more important, there are a number of very pale colours involved, and these are off the page. Where interpolation seems possible, the resulting score is placed in square brackets, e.g. 3 8° [19] means that the page and row are determined, but there is no actual colour square printed in column 19 for row 8°, and the value is arrived at by interpolation between the printed squares.

Since in some of the shells there is a change of colour, only shells of more than 8 mm maximum diameter were used for scoring, inspection showing that any change had occurred by this size. Only intact bivalve shells were used from the samples collected by A. J. C. and J. T. C. since they were abundant (many in fact still with flesh enclosed) although it would be possible to use only left-hand or only right-hand valves in areas where the species is uncommon. Hav-

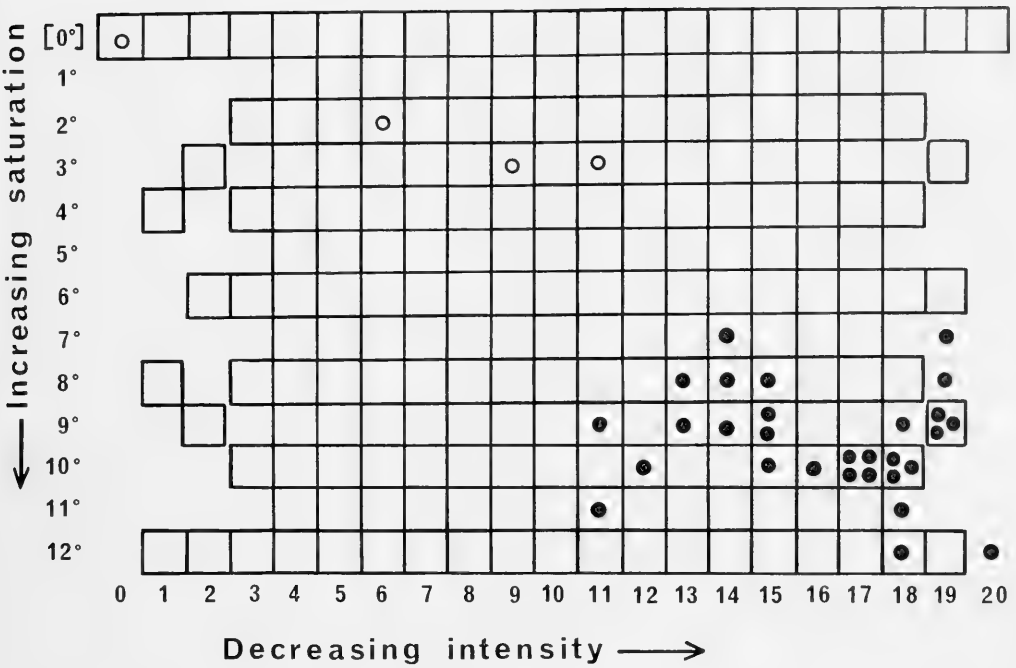

FIG. 1. Colour atlas page summarizing intensity and saturation scores for *Macoma balthica* shells (black circles) and for muddy sand and mud (white circles), irrespective of hue. Colour squares actually printed on every page are outined in black. The top row is of pure neutral colours, repeated on every page. Scores in unoutlined squares are estimated for saturation or intensity or both. *Macoma balthica* colours, irrespective of hue, cluster well away from their sandy mud background colours.

ing both valves means that one can easily recognise small post-mortem stainings which are almost invariably asymmetrical.

(c) Sorting for morphs

Sorting must be done with the shells under water, in a good light. Both the outside and the inside of the valves can become very chalky and appear whitish when dry; wetting ensures that the full colour is seen, and of course gives an appearance corresponding to what is normal in the wild. A few shells were discarded since they were so badly stained black or rust-coloured by burial in the sand, or greened by algae. The true colour can be seen, when there is occasional doubt, by inspecting the muscle attachment scars on the inside of the valve. The shell here is densely glassy, not eroded into chalky patterns by remobilization of its surface calcium during prolonged closures, and gives a useful view into the interior of the shell.

The paired valves were therefore laid out like butterflies in large flat-bottomed trays on a background of white absorbent paper and in about 3 cm of clean water. They were grouped in columns according to hue and intensity so that each column (or series of columns) agreed in hue and progressed from the most to the least intense. Most of the resulting arrangements were checked by J. T. C. Only where there appeared to be a definite discontinuity between adjacent columns was a morph boundary recognised; and all the shells were classified by morphs and intensities before any were colour-scored. This avoided any temptation to break up a continuum of colour variation by assigning it to successive pages of the atlas and produce apparent morphs corresponding to successive pages.

(d) Live material

Some difficulty was found in getting live samples to compare with the empty shells, but one was obtained from the Dee estuary a mile or so up from the Red Rocks collecting points, and some small ones from the

Lancashire coast at Blundellsands. As the shell is somewhat translucent, these were necessary to check on the appearance of the live animal. In fact, the body is mostly translucent white, there being only a slight blackish infusion near the hinge line, presumably corresponding to food or pigment in the digestive gland and perhaps the kidneys. While this dulls the colour of the shell slightly near the umbones it made no difficulty in the scoring, and the score of the empty shell can be accepted as virtually identical with that of the living animal.

(e) Rescoring

Examination of these rather large samples shows at once that there is a true polymorphism, with some remarkable features. Several major colour classes can be readily sorted, as can some intensities of colour within them, and shells with a clear change of colour occurring at about 5 mm maximum diameter. The exact scoring of some of the minor differences in shade of colour is not so easy. Consequently, the major samples were scored on 14 Jan. '84, re-sorted and scored on 15 Feb. '84, and finally scored in Nov. '85, having been put away and not looked at in the intervals. Anyone beginning an investigation of this species should score at first as large a sample as possible, certainly over 300 shells, since some of the colours and colour combinations seem to be rare.

(ii) Notation for morphs and variants

Since *M. balthica* has not been bred, all symbols must be in roman, not italics (Cain, 1987). The most expressive English words for the three shades of red *(sensu lato)* appear, unfortunately, to be purple, pink and peach; they are therefore designated by P_1, P_2, and P_3. Orange O, orange-yellow OY, yellow Y and ivory I are easy to symbolize; the initials of the qualifying words are added for warm yellow WY, chrome-yellow CY, lemon-yellow LY, and warm ivory WI. As W is used for the qualifier 'warm', white is symbolized by Wh.

Nearly all the hues (except white, which is not susceptible of such qualification) vary considerably in intensity. The prefixes VD (very deep), D (deep), M (medium), P (pale) and F (faint) are therefore used as appropriate. A single class, pinkish white (discussed below) is so pale that it is symbolized separately as WhP_1.

For shells that change hue, the same system is adopted, with the initial colour, as seen at the umbones, placed first and separated by a hyphen from the second colour, e.g. PP_1-FO is a pale purple changing to faint orange, MO-DCY a medium orange changing to deep chrome yellow.

(iii) Morphs and variants

The brief descriptions that follow are intended only to indicate the diversity of colour and to assist anyone who is scoring a sample. It would be impossible to print a colour plate with sufficient accuracy to show the exact shades of yellow and ivory, and words plus colour scores can be used to indicate the gaudier forms.

The following list gives the hues in the order of the colour atlas. Each hue is a morph with respect to all the others. Different intensities are probably not morphs but parts of a continuum; possible exceptions are noted. In most shells there is a reduction, with growth, of the intensity of the hue, which is greatest at the umbones (i.e. in the young shells) and decreases somewhat towards the adult shell margin. As the adductor muscle scars are composed of a glassier material than the rest of the shell, they show an intenser hue than the more opaque areas, and indeed in some fresh juveniles (with translucent or nearly transparent shells) produce the effect of two spots of intenser colour even on the outside of each valve. In late juveniles to adults, the valves are nearly opaque, and colour on the outside is confined to the umbones and nearby, and to occasional growth rings which vary in number, placing, and depth of colour from individual to individual. The rest of the outside is a rather dirty white, with pale brown or blackish brown near the margin where the periostracum still remains. The ligament, which is external, appears as a short thick dark-brown line just behind the umbones.

Most shells have only one hue (monochrome). In a small percentage it changes markedly. The change does not correspond, in at least some individuals, and perhaps in all, to a growth line or radius (Fig. 2). So far, only two hues have been seen (dichrome shells). It is possible that the change in intensity usual in monochrome shells may correspond to that from the first to the second hue in dichromes, and that changes from an intense hue to a paler version of the same hue may require special care in scoring. The

FIG. 2. Dichrome shell of *Macoma balthica* 14.2 mm long. The initial colour (outlined on right-hand valve) is about 2 11° 15 (P1) turning rather abruptly to white. The edges of the coloured patch correspond neither to growth lines nor to radii.

slight change in hue occasionally seen between the umbonal region and the muscle scars, which are sometimes slightly less bluish, may be due to the obvious difference in texture of the shell, as noted above, perhaps producing a slight Tyndall blue in the opaquer areas; it might be an actual change of pigment. In the following scores, only the definite changes of hue are noted. These can usually be seen rather faintly on the outsides of the valves.

The colour scores for each hue and intensity are given in Table 5. Taken on the inside of the valve, they give the clearest idea of the actual pigment hue and intensity, and, since morph variation within a single population is far more likely to be genetic than not, they are the best basis for trying to understand the nature of the variation, i.e. polymorphic or continuous. The outside of the valves, however, is presumably what a predator would

TABLE 5. Colour atlas scores for colours of *Macoma balthica* shells.

Hue and intensity	Symbol	Villalobos score	Ridgway equivalent
Very deep purple	VDP_1	2+ 8° 13	XII Amaranth Purple
Deep purple	DP_1	−3 8° 15	XIII Chatenay Pink or XIII Flesh Pink
Medium purple	MP_1	−3 10° 17	XXVIII Shrimp Pink
Pale purple	PP_1	−3 10° 18	XXVIII Shrimp Pink
Faint purple	FP_1	−3 12° 18	XXVIII Shrimp Pink
Pinkish white	WhP_1	−3 12° —	XXVIII Shrimp Pink
Very deep pink	VDP_2	3 9° 11 to 3+ 9° 13	XIII Jasper Pink
Deep pink	DP_2	−4 11° 11	XIII Coral Red
Medium pink	MP_2	−4 10° 16	near XIII Coral Pink
Pale pink	PP_2	−4 10° 18	near XIV Pale Salmon
Very deep peach	VDP_3	5 10° 12	near XIV Cornelian Red or XIV Apricot Orange
Medium peach	MP_3	5 7° 14	XXVIII Japan Rose
Deep orange	DO	5+ 9° 14	XIV Carrot Red or XIV Flesh Ocher
Medium orange	MO	−6 8° 14	XIV Apricot Buff
Pale orange	PO	5+ 10° 18	II Orange Pink
Faint orange	FO	5+ ?9° ?19	—
Deep orange-yellow	DOY	6 9° 15	XV Ochraceous Salmon
Medium orange-yellow	MOY	6 10° 17	III Capucine Buff
Pale orange-yellow	POY	6 11° [18]	near Capucine Buff
Deep warm yellow	DWY	6/7 10° 15	XV Zinc Orange
Medium warm yellow	MWY	6/7 9° 15	near III Capucine Buff
Pale warm yellow	PWY	6/7 9° 19	near III Capucine Buff
Faint warm yellow	FWY	6/7 ? ?	—
Medium chrome yellow	MCY	7/8 10° 17	III Orange Buff
Yellow	Y	8/9 10° 17	III Light Orange Yellow
Lemon yellow	LY	9 ?9° [18]	near XXX Colonial Buff
Faint yellow	FY	9/10 ?9° [19+]	near XVI Straw Yellow
Warm ivory	WI	?7/8 ?7° [19+]	near XV Light Buff
Ivory	I	?8 8° [19+]	near XV Antimony Yellow
White	Wh	−0° 20	—

TABLE 6. Occurrences of hues and intensities in random samples of *Macoma balthica*, Wirral Penninsula.

Sample	V D P1	D P1	M P1	P P1	F P1	Wh P1	V D P2	D P2	M P2	P P2	?V D P3	M P3	D O	M O	P O	F O
(1) Red Rocks. 29 Dec. 83 A. J. C.	4	61	88	12	2	14		6				11	1	7	1	7
%	0.97	14.73	21.26	2.90	0.48	3.38		1.45				2.66	0.24	1.69	0.24	1.69
(2) Red Rocks. 1985 I. Wallace	2	79	48	15		23	12	89	20	13	11	4	7	15	7	6
%	0.34	13.25	8.05	2.52		3.86	2.01	14.93	3.36	2.18	1.85	0.67	1.17	2.52	1.17	1.01
(3) Hoylake 26 Dec. 83 A. J. C.. J. T. C.	8	77	48	8		1		3				6		6		10
%	2.70	26.01	16.22	2.70		0.34		1.01				2.03		2.03		3.38
(4) Hoylake 26 Dec. 83 A. J. C.	8	79	49	12		7	2	1	8			10	4	2	8	10
%	2.31	22.83	14.16	3.47		2.02	0.58	0.29	2.31			2.89	1.16	0.58	2.31	2.89
(5) Hoylake 26 Dec. 83 J. T. C.	3	21	93	15		6		11	6			6		5		7
%	0.89	6.25	27.68	4.46		1.79		3.27	1.79			1.79		1.49		2.08
Totals	25	317	326	62	2	51	14	110	34	13	11	37	12	35	16	40
%	1.26	15.95	16.40	3.12	0.10	2.57	0.70	5.53	1.71	0.65	0.55	1.86	0.60	1.76	0.80	2.01

usually see. In general, the umbonal region outside is the same hue as the inside of the shell but a little paler; the rest of the adult shell is only faintly tinged or plain white except for the remains of the periostracum.

A. Monochromes

1) Purple, P_1

Outside, the umbonal region shows various intensities of a slightly bluish red, a true Tyrian purple (not violet). Inside, the shell is more or less uniformly purple. The range of intensity is considerable (Table 5), from 13 to more than 18, giving very deep, deep, medium, pale and faint purple shells. The shells called pinkish white are placed here because although they are only recognisable by careful comparison with ivories and whites and the colour (internal only) is so faint that it is not easily scorable, they do not seem to belong with other hues.

A careful scoring of shells may perhaps show a discontinuity between MP_1, PP_1 and FP_1, but the distribution of numbers in the samples (Table 6) so far suggests a more or less normal distribution of intensities.

2) Pink, P_2

The hue is noticeably a more yellowish red than in P_1, more obviously so on the inside than on the outside of the shell. In all but sample 2 (Table 6) only a few have been seen; in that sample, the distribution again suggests a continuum rather than discrete morphs.

3) Peach, P_3

Both the umbonal region outside and the inside are a definite peach-colour (colour of peach fruit, not flower), yellower again than P_2. Only small numbers have been seen in any sample.

4) Orange, O

A distinctly orange hue, both inside and out. Although only a few shells have been seen, there is a marked variation in intensity, though not nearly the full range.

5) Orange-yellow, OY

Although intermediate between (4) and (6), these seem to segregate clearly, again with a good range of intensity.

6) Warm yellow, WY

These occur only in Dr. Wallace's sample,

D OY	M OY	P OY	D WY	M WY	P WY	F WY	D CY	M CY	Y	LY	F Y	WI	I	Wh	Dichromes	Total
	8	27						3	4	1	5	20	48	56	28	414
	1.93	6.52						0.72	0.97	0.24	1.21	4.83	11.59	13.53	6.76	
5	9	13	3	6	7	5	1		3	3	24	9	64	53	40	596
0.84	1.51	2.18	0.50	1.01	1.17	0.84	0.18		0.50	0.50	4.03	1.51	10.74	8.89	6.71	
	18	20						3	2		5	12	28	18	23	296
	6.08	6.77						1.01	0.68		1.69	4.05	9.46	6.08	7.77	
	2	28							4		3	18	35	24	32	346
	0.58	8.09							1.16		0.87	5.20	10.11	6.94	9.25	
	15	10						4	10		1	12	63	27	21	336
	4.46	2.98						1.19	2.98		0.30	3.57	18.75	8.03	6.25	
5	52	98	3	6	7	5	1	10	23	4	38	71	238	178	144	1988
0.25	2.62	4.93	0.15	0.30	0.35	0.25	0.05	0.50	1.16	0.20	1.91	3.57	11.97	8.95	7.24	

no. 2 in Table 6, but segregate in it from O, OY and the various forms of Y, again with a considerable range of intensity although in a total of only 21 shells.

7) Chrome yellow, CY

Very few have been seen, 11 in monochromes, and the assignment of one of these to DCY is tentative but supported by variation in the dichromes, in which the second colour appears to be MCY in 7 and DCY in 1.

8) Yellow, Y

Pure yellow. Very little variation in intensity has been seen (see below under (10)).

9) Lemon yellow, LY

Markedly more towards yellow green than (8). Only 4 shells seen, little variation in intensity.

10) Faint yellow

Difficult to score, falling between two pages of the atlas. It is natural to wonder whether this and the last three hues are not merely due to differences in intensity of the same pigment. From the colour atlas this is not the case, as they cannot be matched to different intensities on the same page, and are therefore best regarded as separate morphs. The slight variation in intensity in (7), (8), (9) and (10) is probably due only to the low numbers seen. Usually (Table 6) the medium intensity is the commonest in each hue.

While (10) has been labelled FY, the higher luminosity of yellow as a hue, compared with purple, for example, makes direct comparison with the others difficult. Perhaps a separate colour-designation and symbol should be used.

11) Ivory, I

This group, so pale that its exact hue and page in the atlas are dubious, segregates quite clearly from the hues above and from white. Warm ivory also seems to segregate, but it may be an extreme of ivory. Their relative numbers in Table 6 do not exclude this possibility.

12) White, Wh

This is a good plain white, segregating well from the ivories and other colours, except that careful comparison is needed to separate pinkish white WhP_1 (see under (1) above). It appears to be produced by the absence of pigments, and therefore has no variation in intensity.

TABLE 7. *Macoma balthica* dichrome shells in Wirral samples. Sample nos. as in Table 6.

	(1)	(2)	(3)	(4)	(5)	Total
VDP_1-MP_3			2			2
DP_1-FY					1	1
MP_1-MP_3	6					6
MP_1-PO	5		1	3	1	10
MP_1-MCY	1					1
MP_1-Y	3			1		4
MP_1-FY	2					2
MP_1-I		1				1
MP_1-Wh	1	1	1			3
PP_1-MO		6			6	12
PP_1-PO	5		7		5	17
PP_1-FO					2	2
PP_1-POY		1		8		9
PP_1-DWY		2				2
PP_1-MWY		3				3
PP_1-MCY				3		3
PP_1-Y		1		2	2	5
PP_1-LY		1		1		2
PP_1-FY					3	3
PP_1-WI		7		1		8
PP_1-Wh	1	4	7	1	1	14
FP_1-MWY		6				6
FP_1-MCY	2		1			3
FP_1-FY				3		3
FP_1-WI		2				2
FP_1-I		1				1
FP_1-Wh	2	2	4	8		16
PP_3-LY		1				1
MO-DCY		1				1
MO-I				1		1
Total	28	40	23	32	21	144
% in sample	6.76	6.71	7.77	9.25	6.25	7.24

B. Dichromes

The full list of dichromes seen is given in Table 7 with their occurrence in the samples. So many are represented by one, two or three shells (19 out of 30) that doubling the size of the samples might well double the number of sorts observed. If it is considered that different intensities of the same hue are parts of a continuum, we can lump the sorts in Table 7 by ignoring the variation of intensity of both the first and second colours; there still remain 11 distinct dichromic types. The total frequency of dichromes in each sample is always low (Table 7; mean 7.24%), but might be raised by very careful scoring of apparent monochromes.

The full scores according to the scheme given above of all the Wirral samples are given in Table 6, with the details of dichromes in Table 7. Dr. Mallet's collection from near Camber Sands is of drifted single valves, somewhat worn. To avoid scoring the same individual twice, the valves were sorted by chirality as well as into colours, and the largest number, whether of left-hand or right-hand valves, was taken as the score for each colour. The Baltic specimens could only be scored from the outside. The scores for both are shown in Table 8. The lack of dichromes in the Baltic sample may or may not be genuine, but the lack of yellows probably is.

(iv) Features of the polymorphism in Wirral samples

The scores given in Tables 6 and 7, and the colour scores for the different hues in Table 5,

TABLE 8. Scores of samples of *Macoma balthica* from non-Wirral localities.

	Purple, pink	Peach	Orange	Pale orange	Orange-yellow	Yellow	Ivory, white	Dichromes	Total
A nr. Camber Sands									
J. Mallet	64	15	28	12		18	62	20	219
%	29.22	6.85	12.79	5.50		8.22	28.31	9.13	
B Baltic coast									
G. Russell	52	———	8	———			407		467
%	11.13		1.71				87.15		

bring out a number of points with regard to the polymorphism.

(a) Although the range of hues is wide, from purple through peach, orange and various yellows to ivory, it is continuous (atlas pp. 2 to 10) and restricted when compared with the full range of possible colours, only 9 pages out of 38. It cannot be said that this is due to an incapacity of molluscs, or even bivalves, for producing greens, blues and violets. Dark blues and occasional purples are well known in some mussels (e.g. *Mytilus edulis, Modiolus modiolus*) and greens in others *(Musculus).* Brown, as rays or mottles, is conspicuous in *Glycymeris glycymeris, Mactra corallina* and *Venus striatula.* A rich purple is seen inside the valves of *Gari fervensis,* violet in *Donax vittatus.* Numerous other examples could be given from foreign bivalves.

The general area of muddy sand scores on the colour charts (Fig. 1) is around 7 4° 10, extending from thence towards 1° 1 as it gets blacker with contained mud. (At such dark colours as these, the page number can vary widely with little effect). The general ensemble of the shell colour scores, projected on to the same page is about as far from the muddy-sand and mud scores as it can be (Fig. 1). It is obvious, and equally so from a glance at the live animals, that there is no trace of cryptic coloration here. It is interesting to contrast with *M. balthica* the common cockle, *Cerastoderma edule,* often found with it or nearby. Large juveniles and adults have a white shell, very strongly constructed, and easily seen like the ivory and white morphs of *M. balthica,* but very small juveniles usually have a scattering of dark markings (rows of short dashes) which effectively mottle the shell and, make it less conspicuous.

If the coloration is not cryptic, since it is markedly diverse it is possible that we have here a form of apostatic polymorphism (Clarke, 1964). But if so, the coloration is not diverse enough for a fully apostatic polymorphism. It is easy to predict from the atlas what such a polymorphism should be. If n is the number of morphs observed, and any one taken at random is found to fall on a particular page of the atlas, then the others should be spaced as far from it and each other as possible. They will occupy the vertex positions of a polygon, inscribed within the cylinder of pages (Fig. 3), with number of sides and of vertices equal to n, one of the vertices being located at the page with the observed score for a morph. Thus, if we take it that there are three principal morphs, one of which is purple, scoring on p. 2, the other two should be at the vertices of an inscribed equilateral triangle within the circle (Fig. 3), i.e. near to p. 15 (lime-green) and 27 (cobalt-ultramarine). Purple-pink is in fact one principal class of hue, but the others actually observed are orange, p. 5–6, and yellow, p. 8+ to 10−. They are approximately equally spaced but far too close together for pure apostatic selection. A further separation could be made by alternately displacing the colour score up and down the pages. If orange is lowered in its intensity score, a rich brown results. Lowering the score for yellow would produce a dull green. Neither of these is found. Raising either would produce pale

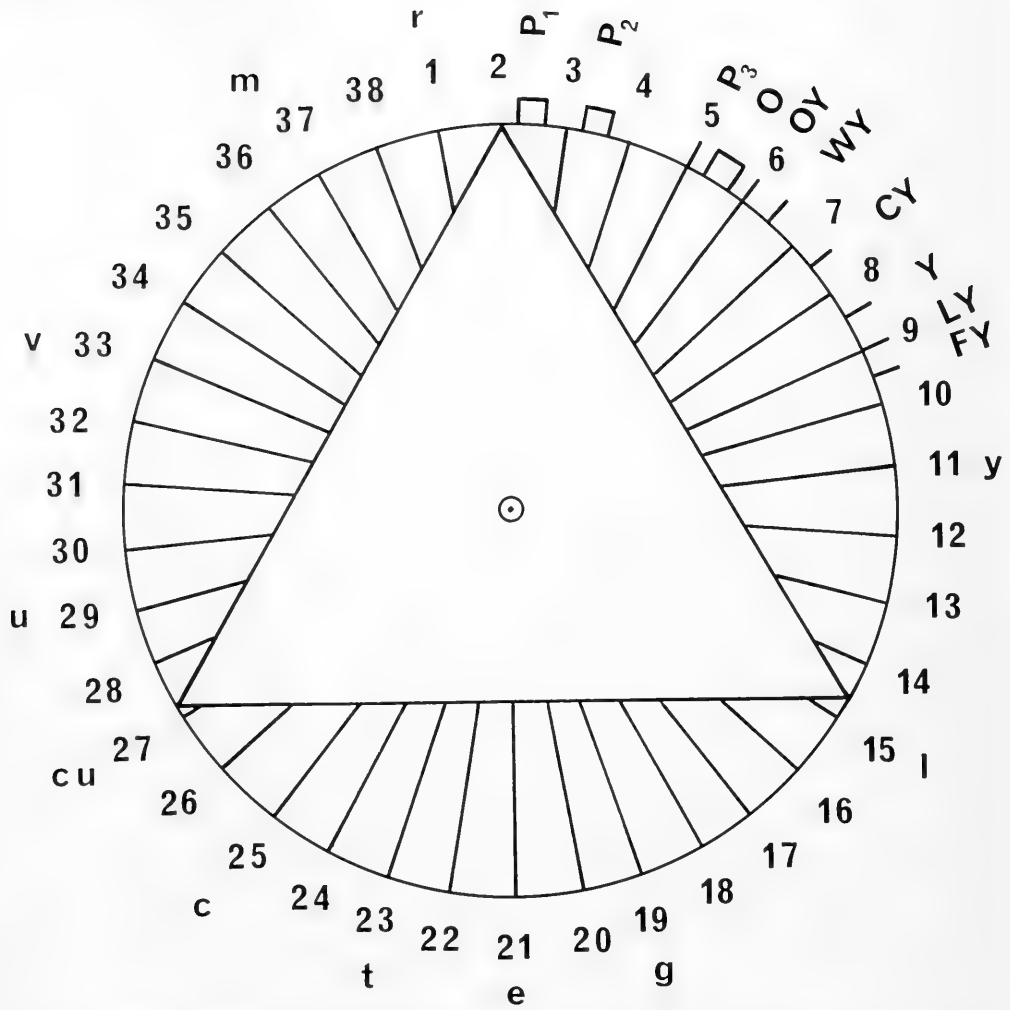

FIG. 3. Predicted and observed hues of morphs of *Macoma balthica* on the hypothesis of apostatic selection acting alone. The 38 radii are the edges of the pages of the Villalobos colour atlas grouped as half-sections of a cylinder with the neutral colour row (common to all pages) as the central axis. Observed hues labelled with morph symbols (P1, OY etc., see Table 5). They fall into 3 major groups, purple-pink, orange, and yellow. If purple-pink is taken as the datum, then under plain apostatic selection the other two hues should be as far apart from it and each other as possible. White is also a morph but is not allocatable to any hue; if shown, it will be at the centre of the circle. The vertices of the inscribed equilateral triangle imposed on the circle give the predicted positions of non-white hues; these are close to lime green (1, p. 15) and cobalt-ultramarine (cu, p. 27). P. 3 is scarlet, s, p. 7 orange, o, in the Villalobos terminology, which is marked for the principal hue-names by lower-case letters.

colours, but these would then approximate to whites.

(b) For apostatic polymorphism, not complicated by various degrees of crypsis (as in *Cepaea,* Cain, 1977, 1983) not only should the hues be as diverse as possible, they should also differ visibly as much as possible. There should be no admixture of neutral colour, which would render them more similar, and all should score the maximum (12°) for saturation. While most are duller than this (Table 5) almost all approximate to that edge of the page. The dullness probably means only that the colour is not displayed on a pure white background. Equally, the most brilliant intensity should be used in all, since great intensities produce very similar browns, olives etc., and lesser ones produce pale colours,

approximating to white. Here we find a marked discrepancy (Fig. 1). To the eye, hues of saturation 12° and intensities between 10 and 14 make the most noticeable difference in colour from page to page. Yet (Table 5) in *M. balthica* the same hue, e.g., P_1, ranges from above 10 to beyond the limits of the page; this is true for P_1, O, WY and one of the paler yellows, and nearly so for P_2. Probably larger samples would extend the range for other hues less abundantly represented so far. This variation towards white would in itself argue against simple apostasy, but it is the more remarkable in that white and ivory (themselves strikingly alike) are abundant morphs in all the samples, and these pallid hues converge on them.

(c) This tendency to similarity is taken much further when we look at the outsides of the shells. Small juveniles have glossy translucent or nearly transparent shells which show their hue and intensity almost as well externally as internally. On larger shells the umbonal region continues to show the colour of the juvenile, but the rest of the shell is an opaque greyish white, relieved only by the pigmentation of occasional growth lines, seldom as intense as the umbonal colour, and the remains of brown periostracum near the growing edge. Only a faint general tinge of the internal hue is visible outside. Juveniles are very diverse, other age-groups less so, although it is true that the eye is caught by the bright umbonal colours forming two patches sharply divided posteriorly by the conspicuous brown ligament.

(d) Some of the colour-classes may be produced by combinations of the pigments mediating the principal colours. This is certainly the case in populations of the winkle *Littorina rudis* (Maton) that I have examined, in which at the mouth of some shells two pigments can be scored because their distributions do not quite overlap. In the present polymorphism, no such convenient separation has been observed, and it cannot be asserted that the orange-yellow class, for example, is in fact made by the suffusion of the shell by both the yellow and the orange pigments, but it may be; and if so, presumably it is heterozygous, with no dominance, for the two colours.

(e) In some forms two pigments are certainly present but they do not overlap. As the shell grows there is a fairly rapid switch from one to another. In almost every case, the change is from a redder to a yellower or whiter hue. In *Cepaea,* colour heterozygotes not infrequently begin with the recessive colour and then rapidly change to the dominant, so that the recessive is only seen close to the apex of the spire (only very rarely have I seen shells that changed much later). In *Macoma balthica* the change is regularly at about ¼ of the final area of the shell.

A number of these dichrome shells, especially those changing to pale yellow or white, hardly differ from monochromes of the same first (umbonal) hue since these are usually white externally except for the umbonal areas.

(v) Frequencies of morphs in Wirral samples

Many of the cells in Table 6 would give expectations of less than 5 for a χ^2 test. The least further grouping that would give a probably valid test is VDP1 + DP1, PP1 + FP1, VDP2 + DP2, MP2 + PP2, DP3 + MP3, DO + MO, PO + FO, DOY + MOY, WY + DCY + MCY, Y + LY + FY; all expectations are more than 5 except one, sample 3, WY + DCY + MCY, which is 4.76. This grouping shows a highly significant χ^2 (d.f. 64) = 476, $P << 0.001$. Separation by locality into (1) + (2), Red Rocks, and (3) + (4) + (5), Hoylake, necessitates the further grouping of the Hoylake samples, namely PP1 + FP1 + Wh1, all P2, all O, and all Y after POY. Both sets of samples still show highly significant differences, as follows.

Red Rocks χ^2 (d.f. 16) = 153, $P << 0.001$.
Hoylake χ^2 (d.f. 24) = 121, $P << 0.001$.

In the Red Rocks samples, the major differences are in the proportions of MP1 and VDP2 + DP2, POY, and WI. As the proportions of the P1 groups other than MP1 are in good agreement, it seems unlikely that a mistake has been made in scoring the intensity of pigmentation; the same is probably true of VDP2 + DP2, since the other P2 group (MP2 + PP2) differs only by 5%, and similarly of the OY groups and WI against I.

In the Hoylake samples, there is a marked discrepancy in both VDP1 + DP1 and MP1 in sample (5), deficiencies in DOY + MOY in sample (4) and in POY in sample (5), and an excess of I in (5).

Since the shells are from a shell-wreck, not collected alive *in situ,* it is difficult to suggest biological reasons for these discrepancies.

No shells were perforated by *Natica* or similar predators, nor were any single valves collected, so that the reasons for differential drifting demonstrated experimentally by Lever (1958; Lever *et al.*, 1961, 1964) do not apply. It is conceivable that different samples have different proportions of intensities of hues because there is some fading with time, or indeed that the exact intensity produced in a given shell is partly dependent on its conditions of growth and therefore on weather or other environmental factors.

What are unlikely to be affected by either of these factors are the exact hues, most of which seem to be true morphs. Regrouping under each locality by hue still gives highly significant differences for the two Red Rocks samples; the three Hoylake ones are now just not significant. A further grouping into all pinks, all oranges (including OY), all yellows, and all whites (I + Wh) produces the same results.

Regrouping by effective hue requires the transfer of FP1, WhP1, FO, FWY and FY to a separate group comprising also WI, I and Wh, and to obtain expecteds greater than 5 requires grouping by hue. Again, samples 1 and 2 are highly significantly different, 3, 4 and 5 are on the borderline of significance ($P = 0.01$).

Since the Wirral samples except no. 2 (Dr. Wallace's) are taken from the same shell-wreck, it is probably better biologically to regard them as different subsamples from the same huge sample. As such, they agree well with one another (Tables 6, 7). Dr. Wallace's sample was taken later from the same area of beach as no. 1. It differs strikingly in the nearly equal proportions of P_1 and P_2 instead of a vast preponderance of P_1 over P_2, and in the presence of WY (absent in the other samples). This last, and an apparently high proportion of FY (4% instead of about 1%) are compensated for to a large extent by the relatively low proportions of OY (4 as against 9%). The significance of these differences is considered further in relation to the south coast sample.

If we group the hues into P, O, OY, WY + CY + Y, LY + FY, I, Wh, and all dichromes, the Wirral samples are so similar that they can be combined and give the following percentages:

P	50.40	LY etc.	2.11
O	5.18	I	15.54
OY	7.80	Wh	8.95
WY etc.	2.77	Dichromes	7.24

By far the commonest are the pinks (broad sense), with ivories and whites well behind (together, 24.49) and the various oranges and yellows making up 17.86%. In actual appearance, however, the various faint intensities and pinkish white should be allocated with ivory and white, giving a percentage of effective white overall as 31.33 (range in samples, 25.00 to 34.52), as against 24.49. In practice, looking at actual samples on the shore, there is no doubt of the visual effect—the majority are pinks (broad sense) and whites, with a sparse scatter of dichromes, yellows and oranges. The finer distinctions that can be made on a sample of cleaned shells are largely obliterated. Since many shells are partly obscured by sand or mud, and often show growth-line variations in intensity of colour, the dichromes are not very conspicuous, and the whole, to a casual eye, becomes a scatter of pinks and whites, with a very few yellows markedly conspicuous. To a careful observer, including no doubt predators, distinctions are much more obvious.

(vi) Non-Wirral samples

Dr. Mallet's sample from near Camber Sands, A, being somewhat waveworn, and that from the Baltic, B, not being removable from the bodies, less fine divisions into hues have been used; the scores are given in Table 8. As compared with the Wirral samples, A is somewhat low in pinks (30% as against 45%), high in orange (13% vs. 3–6%), deficient in orange-yellows (absent), high in yellows taken together (8% vs. 3–4%), and very similar in ivory + white and dichromes. In its high values of orange and yellow it approaches sample 2 (Dr. Wallace's) but this last was collected by scooping, not by hand-picking. Otherwise, the general agreement of A with the Wirral samples is good.

The Baltic sample, in complete contrast, is 87% white, 1.7% orange and 11% pink (all hues taken in the broad sense). The pinks give the impression of being very uniform, much more so than in the English samples. Dr. Russell tells me that, at the place of collection, the bottom was largely covered with a whitish calcareous deposit. This is the only sample that could be protectively coloured. Smith (1975) noted that in *Donax faba* (Gmelin) the very small shells are about the size of the sand grains of the substrate and, scattered among them, may be hard to see because of their diversity of colours. The

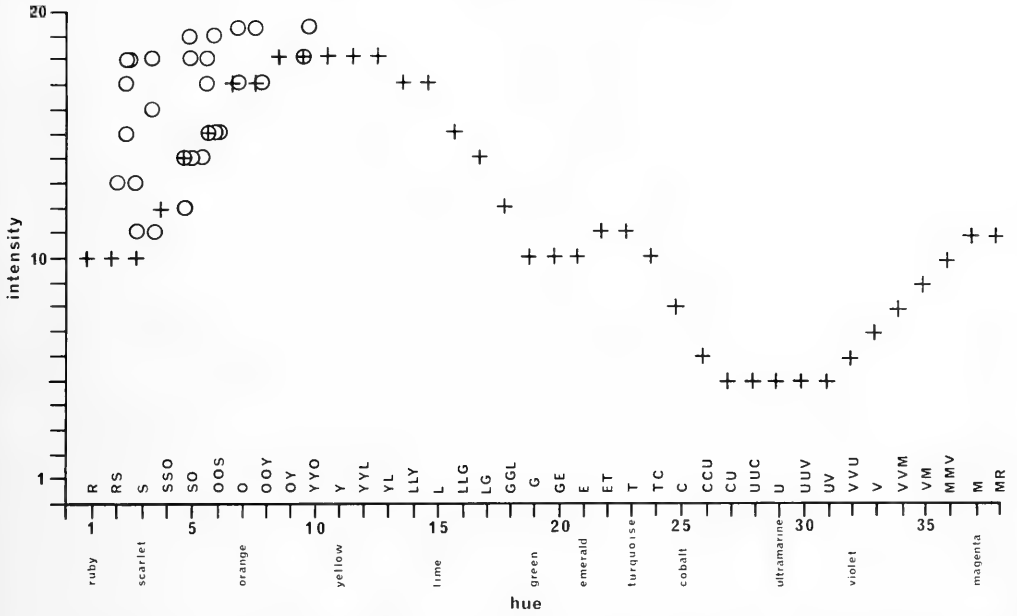

FIG. 4. Variation of intensity of generating square for each hue in the Villalobos colour atlas; in effect, variation of luminosity of hues (crosses). Orange, yellow, and yellow-green are the most luminous. Colour scores for hue and intensity of *Macoma balthica* morphs and variants (open circles) show a close correspondence to the generating intensities but also a considerable scatter of paler colours. White shells are not allocable to any hue.

muddy sand on which *M. balthica* is found appears to be finer in texture, and it is unlikely that small juveniles would be cryptic, although new-fallen spat possibly might.

(vii) Frequencies and apostatic selection

As pointed out above, except for the Baltic sample none of the hues and intensities come near to those of the background against which *M. balthica* would be seen. If all the morphs and forms were equally conspicuous on a given background and subject to apostatic selection, one would expect the frequencies of all to be equal at equilibrium. If not, less conspicuous ones will be at a frequency higher in proportion to their degree of crypsis, at least to a first approximation.

Equivalent molar concentrations of different pigments give different intensities of colour. It is noticeable in the Villalobos atlas that the colour-square on each page giving the colour from which the rest of the page is generated by concentration, or dilution with transparent medium, or dilution with neutral tint, varies rather regularly with hue (Fig. 4) such that in the sequence of colour used by *M. balthica,* at equivalent concentrations reds cut out more light than oranges, these than yellows, and these, of course, than ivories and whites. The intensest hues observed so far in *M. balthica* give intensity values of approximately

Pink	Peach	Orange
14	15	17
Chrome	Lemon	Ivory
18	19	unscorable

(all of which, of course can appear as fainter dilutions), while wet muddy sand and mud are from about 10 at the very highest to 0 (probably usually below 6 if the substrate carries live *M. balthica*). Their colours are deep browns, black-browns and near-black, often of very indeterminate hue. Wet pure sand, of the sort usually described as yellow, falls about p. 7, which is between chromes and lemon yellows, so that its generating hue is within the range of colours seen in *M. balthica,* but is much darker than they are; and in fact yellows stand out, to the human eye, very conspicuously on dirty sand, let alone mud.

Comparing pages in the atlas, the distinction between hues is most immediately recognisable in the purest colours (12°) between about 12 and 17, those below being rather dark, those above too dilute, pale and washed-out. To a predator with colour-vision, therefore, different colour morphs should look most distinct at these intensities.

In fact, colour scores (Table 5) in this range are shown by the deep and medium intensities. The darkest are purple, pink and peach, and these, therefore, are closest to sandy mud in intensity. White and the palest colours are obviously furthest away. If we arrange the hues in increasing intrinsic paleness, this should be the ranking in decreasing frequency if all hues are equally conspicuous but intensity is acted on by apostatic selection.

Table 6, which is in this order of increasing luminosity of hue (not intensity) immediately shows that the hypothesis is untenable, since the most abundant classes are at both ends of the distribution. Regrouping by effective hue, i.e. removing FP_1, WhP_1, FO, FWY, and FY to the white group (WI, I and Wh) decreases the frequencies of purples and pinks and increases that of white which should be the least. Fig. 5 gives frequency distributions of the frequencies, for internal or effective scores, lumped by hue or completely separate, dichromes being omitted as heterogeneous. None approximates to a scatter around the expected value.

If colour is supposed to have any cryptic effect, then OY and WY are the likeliest to approximate to that of sand. They are not commoner than the less cryptic colours in any sample.

(vii) Biology of Macoma balthica

As an easily identified and often exceedingly abundant species, *M. balthica* has been used often for research, both ecological and physiological; the large literature can be approached through the references in Beukema & Meehan (1985), Brafield (1963), Brafield & Newell (1961), McLusky & Allan (1976), Meehan (1985), and Meehan & Diaz (1984). As so often with well-known, abundant and variable species, the taxonomy is only now beginning to be worked out properly; there are strong indications (Meehan, 1985; Beukema & Meehan, 1985) that eastern North American populations (and presumably western North American also) are not conspecific with the European *M. balthica*.

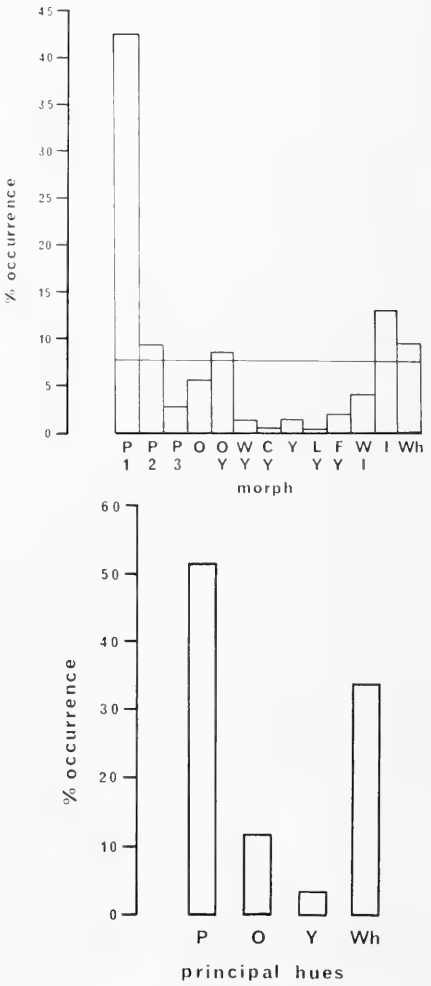

FIG. 5. Frequencies of hues plus white in the combined Wirral samples of *Macoma balthica,* in order of increasing luminosity, the few dichrome shells being omitted.

5a, percentage occurrences of all morphs, data from Table 6. If all morphs are equally conspicuous and apostatic selection is acting, the expected percentages are given by the horizontal line at 7.69%. Since hue and intensity vary together (fig. 4) if intensity is being selected (e.g. by colour-blind predators), the percentages should decrease steadily from left to right, the pink morphs being darker and more like the background. If any hue is cryptic it should be the yellow class, and percentages should decrease from it in both directions.

5b, faint colours and pinkish white lumped with white, and correspondingly all pinks, all oranges, all yellows and all effective whites lumped. The only result is to emphasize the bimodality of the percentages, unexpected on any of the three hypotheses just given.

Of particular importance for the present work is the occurrence of *M. balthica* largely between the tidemarks, also extending just below but usually most abundant at the mid-tide level (Brafield & Newell, 1961) and so very much exposed to bird predators when the tide is out. It occurs in mingled sand and mud or actual mud (Yonge, 1949; Brady, 1943; Brafield & Newell, 1961) being largely replaced in clean sand by *Tellina tenuis* (Yonge, 1949; Barrett & Yonge, 1958); it is especially abundant in estuaries (e.g., Brady, 1943; Stopford, 1951). When the tide is out it is found at depths from the surface to 12 cm but can be seen burrowing through wet substrate (Stopford, 1951). Brafield (1963) shows that anoxic conditions in the substrate cause it to rise to the surface where it can breathe oxygenated water lying in ripple hollows, and Brafield & Newell (1961) document its moving about feeding on the beach and leaving furrows of various shapes in the substrate, straight, U-shaped or nearly circular.

The siphons are highly extensile, and when the tide is in, *M. balthica* may feed with the body at several cm depth in the substrate, sucking up detritus on the substrate surface (deposit feeding) or if suspended food is available, taking that. Brady (1943) suggests that its ability to do both allows it to compete with obligatorily deposit-feeding polychaetes. Newell (1965) has investigated its feeding on detritus.

Such data as these make it clear that *M. balthica* is very much at risk from visual predators (birds) when the tide is out; fish may also be important when the tide is in. Although many fish predators take only the siphons, or, as with flatfish, use touch not sight in their foraging, some at least may snatch out individuals in the top layer of the substrate.

In an important paper, Beukema & Meehan (1985) survey the distribution of shell colour in *M. balthica* on both sides of the Atlantic, using the broad categories red, orange, yellow and white, and noting the occurrence of a few percent of bicoloured shells "mostly either white or yellow with a red spot near the umbo". There are marked differences between the eastern American and the European populations. In Europe, as a result of extensive collections, they note that red tends to be most abundant in the north, yellow is largely confined to France, and the proportion of white is highly variable. They make the intriguing observation that in Europe "The share of these uncoloured shells appeared to be particularly high in brackish waters (as in the Baltic), but was also more than about 0.6 at some sampling places with a salinity near 30‰. In very muddy sediments the proportions of indistinctly coloured shells was often high but not necessarily so." "Shell colours were most vivid in samples from sandy (i.e. exposed) places; samples from muddy sediments contained high proportions of whitish, greyish or bluish shells; at times, to such an extent that the colour sorting of the sample had to be abandoned."

My visits to numerous coastal localities in the British Isles and Brittany, and some in the Bay of Biscay (French and Spanish) and the French Mediterranean coast do not suggest that there is any special yellowness in the sand substrates in France as against those in Britain, whereas there is in France a marked development of chrysophycean algae that colour a band of rocks on the shore and affect the colour of the coincident *Littorina* species. The Baltic sample reported here agrees entirely with Beukema & Meehan's remarks, as do the others which are from sandy mud rather than only mud (probably including the Camber sands sample when alive). Beukema & Meehan comment on the diversity of shell colour "nor can we easily suggest a possible adaptive significance, as the growing individuals disappear below the sediment surface as soon as the shell colours develop".

DISCUSSION

It was concluded tentatively (p. 295 above) from the general survey of British bivalve coloration in relation to probable exposure that it supports the expectation that the external colours and patterns of marine bivalves are strongly influenced by visual predators. Even infrequent exposure, or exposure mainly as the juvenile, may in time produce a very considerable selective effect.

The biology of *Macoma balthica* (p. 308) certainly suggests that this species is open to considerable pressure by visual predators, perhaps even more to terrestrial than to aquatic ones. The vision of its principal predators is not likely to be influenced by depth of water and consequent filtering of colours. Its display of a true colour polymorphism with no morphs resembling its background immediately suggests apostatic selection. However, various features of the actual morphs, namely

the narrow range of hues, production of very pale forms of most hues resembling the common white morphs, and the relative abundances of different morphs all raise difficulties on the supposition of pure apostatic selection. The very different distributions of major colour classes in Europe recorded by Beukema & Meehan (1985) might suggest some sort of climatic selection with darker morphs in the northern parts of the range, and perhaps even with white forms more common on the blacker backgrounds which would heat up more during neap tides in the summer. If this is true, selection should be stronger on the juveniles which, with their shorter siphons, would need to stay closer to the surface of the substrate. A further suggestion is that the animals are mimicking their own dead shells which often lie about in great abundance, and are useless for food; but on this supposition, all the dead shells should be alike. It is conceivable that the small juveniles, much more vulnerable to predators, are showing an apostatic polymorphism and later become white for this very reason; this would not involve group selection. In this case the retention of colour inside the shell seems to be a functionless continuation of the juvenile colour.

All such suggestions as these can only be tested by a full investigation into the predator-prey relationships of the species, especially in the young stages, with special attention to low frequencies of predation, the habits and modes of search of different predators, and especially the habits of the prey. More bivalves than yet suspected may be quite athletic, at least when juvenile. Speculation is useful only as it suggests ideas to work on. While no doubt paragraphs could be devoted to possible influences of developmental constraints, linkage disequilibria, and the like, the total absence of any data on such matters suggests that they be postponed.

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APPENDIX A. British marine bivalves: types of coloration and probable degree of exposure.

The types of colour and pattern variation (I–IV) and the probable degrees of exposure ((i)–(iii)) are defined in the text, section II (iii) and II (vi).

Species and classification	Maximum measurement (cm[12])	Type of colour and pattern variation	Probable degree of exposure
BIVALVIA[1]			
PALAEOTAXODONTA			
Nuculoida			
Nuculacea			
Nuculidae[2]			
Nucula sulcata Bronn	1.90	II	(ii)
Nucula nucleus (L.)	1.27	III	(ii)
Nucula hanleyi Winckworth	1.27	II	(ii)
Nucula turgida Leckenby & Marshall	1.27	II	(ii)
Nucula tenuis (Montagu)	1.27	III	(ii)
Nuculanacea			
Nuculanidae			
Nuculana minuta (Müller)[2]	1.90	III	(ii)
Yoldiella lucida (Lovén)	0.32[m3]	III	(ii)
Yoldiella tomlini Winckworth	0.48[m]	III	(ii)
Phaseolus pusillus (Jeffreys)	0.16[m]	III	(ii)
(CRYPTODONTA)			
(Solemyoida)			
(Praecardioida†)			
PTERIOMORPHA			
Arcoida			
Arcacea			
Arcidae			
Arca tetragona Poli	5.08	III	(i)
Arca lactea L.	1.90	IV	(ii)
Arca pectunculoides Scacchi	0.48[m]	IV	(ii)
Limopsacea			
Glycimeridae			
Glycimeris glycimeris (L.)[4]	6.35	II	(ii)
Limopsidae			
Limopsis aurita (Brocchi)	1.27	IV	(ii)
Mytiloida			
Mytilacea			
Mytilidae			
Mytilus edulis L.[5]	15.24	II	(i)
Modiolus modiolus (L.)	22.86	II	(i)?
Modiolus barbatus (L.)	6.35	II	(i)?
Modiolus adriaticus Lam.	3.81	II	(i)?
Modiolus phaseolinus (Philippi)	1.90	II	(i)?
Adula simpsoni (Marshall)	1.90	III	(iii)
Musculus discors (L.)	1.27	II	(i)?
Musculus marmoratus (Forbes)	1.90	II	(i)?
Musculus costulatus (Risso)	1.27	II	(i)?
Musculus niger (Gray)	5.08	II	(i)?
Crenella decussata (Montagu)	0.32[m]	III	(i)?
Crenella prideauxi (Leach)	0.32[m]	III	(i)?
Pinnacea			
Pinnidae			
Pinna fragilis Pennant	30.48	II	(i)?
Pterioida			
Pteriina			
Pteriacea			

APPENDIX A (Continued)

Species and classification	Maximum measurement (cm[12])	Type of colour and pattern variation	Probable degree of exposure
Pteriidae			
Pteria hirundo (L.)	7.26	II	(i)
Pectinacea			
Pectinidae			
Pecten maximus (L.)[4,6]	15.24	I	(i)
Chlamys sulcata (Müller)	2.54	I	(i)
Chlamys varia (L.)	6.35	I	(i)
Chlamys nivea (Macgillivray)	6.35	IV	(i)
Chlamys distorta (da Costa)[6]	3.81	I	(i)
Chlamys opercularis (L.)	8.89	I	(i)
Chlamys septemradiata (Müller)[4]	5.08	I	(i)
Chlamys tigerina (Müller)	2.54	I	(i)
Chlamys furtiva (Lovén)	1.90	I	(i)
Chlamys striata (Müller)	1.90	I	(i)
Chlamys vitrea (Gmelin)	1.90	IV	(i)
Similipecten similis (Laskey)	0.95	I	(i)
Anomiacea			
Anomiidae			
Anomia ephippium L.	6.35	II	(i)
Monia patelliformis (L.)[8]	3.81	III	(i)
Monia squama (Gmelin)[8]	3.81	III	(i)
Heteranomia squamula (L.)	1.27	III	(i)
Limacea			
Limidae			
Lima hians (Gmelin)[9]	2.54	IV?	(i)??
Lima loscombi Sowerby	1.90	IV	(ii)
Lima sulcata Brown	1.27	IV	(ii)
Lima subauriculata Montagu	0.63	IV	(ii)
Lima sarsi (Lovén)	0.32[m]	IV	(ii)
Ostreina			
Ostreacea			
Ostreidae			
Ostrea edulis L.	10.16	III	(i)
Crassostrea virginica (Gmelin)	17.78	III	(i)
Crassostrea angulata (Lamarck)	17.78	III	(i)
(PALAEOHETERODONTA)			
(Modiomorphoida[†])			
(Unionoida freshwater)			
(Trigonioida)			
HETERODONTA			
Veneroida			
Lucinacea[10]			
Lucinidae			
Loripes lucinalis (Lam.)	1.90	IV	(iii)
Myrtea spinifera (Montagu)	2.54	IV	(iii)
Lucinoma borealis (L.)	3.81	IV	(iii)
Divaricella divaricata (L.)	1.27	IV	(iii)
Ungulinidae[11]			
Diplodonta rotundata (Montagu)	2.54	IV	(iii)
Thyasiridae			
Thyasira flexuosa (Montagu)	0.95	IV	(iii)
Thyasira croulinensis (Jeffreys)	0.32[m]	IV	(iii)
Thyasira ferruginea Winckworth	0.32[m]	II	(iii)
Thyasira subtrigona (Jeffreys)	0.16[m]	IV	(ii)?
(Chamacea)			
Leptonacea			

APPENDIX A (Continued)

Species and classification	Maximum measurement (cm[12])	Type of colour and pattern variation	Probable degree of exposure
Erycinidae			
Lasaea rubra (Montagu)[12]	0.32[m]	II	(iii)
Kelliidae			
Kellia suborbicularis (Montagu)	0.95	IV	(iii)
Leptonidae			
Lepton squamosum (Montagu)	1.27	IV	(iii)
Lepton nitidum Turton	0.32[m]	IV	(ii)
Neolepton sulcatulum (Jeffreys)	0.16[m]	IV	(ii)
Neolepton sykesi (Chaster)	0.16[m]	IV	(ii)
Epilepton clarkiae (Clark)	0.16[m]	IV	(ii)
Montacutidae			
Montacuta substriata (Montagu)	0.32[m]	IV	(iii)
Montacuta ferruginosa[13] (Montagu)	0.79	II	(iii)
Mysella bidentata[4] (Montagu)	0.32[m]	IV	(ii)
Galeommatidae			
Galeomma turtoni Sowerby	1.27	IV	(i)?
Devonia perrieri (Malard)	0.48[m]	IV	(iii)
(Chlamydoconchacea)			
Cyamiacea			
Turtoniidae			
Turtonia minuta (Fabricius)[14]	0.32[m]	II	(iii)
(Carditacea)			
Crassatellacea			
Astartidae			
Astarte sulcata (da Costa)	2.54	III	(ii)
Astarte elliptica (Brown)	3.17	III	(ii)
Astarte montagui (Dillwyn)	1.27	III	(ii)
Astarte triangularis (Montagu)	0.32[m]	III	(ii)
Astarte borealis (Schumacher)	4.44	III	(ii)
Cardiacea			
Cardiidae			
Acanthocardia aculeata (L.)	10.16	III	(ii)
Acanthocardia echinata (L.)[15]	7.62	III	(ii)
Acanthocardia tuberculata (L.)	8.90	II	(ii)
Parvicardium minimum (Philippi)	1.27	IV	(ii)
Parvicardium papillosum (Poli)	1.27	III	(ii)
Parvicardium ovale (Sowerby)	1.27	IV	(ii)
Parvicardium scabrum (Philippi)	1.27	IV	(ii)
Parvicardium exiguum (Gmelin)[16]	1.27	III	(ii)
Cerastoderma edule (L.)[15]	5.08	III	(ii)
Cerastoderma glaucum (Lam.)[17]	6?	III	(ii)
Laevicardium crassum (Gmelin)[15]	7.62	III	(ii)
(Tridacnacea)			
Mactracea			
Mactridae			
Mactra corallina (L.)[18]	5.08	II	(ii)
Mactra glauca Born	11.43	II	(ii)
Spisula elliptica (Brown)	3.17	IV	(ii)
Spisula solida (L.)	4.44	IV	(ii)
Spisula subtruncata (da Costa)	2.54	IV	(ii)
Lutraria lutraria (L.)	12.70	III	(iii)
Lutraria magna (da Costa)	12.70	III	(iii)
Lutraria angustior Philippi	10.16	III	(iii)
Mesodesmatacea[19]			
Mesodesmatidae			
Ervilia castanea (Montagu)	1.27	II	(ii)
Solenacea			

APPENDIX A (Continued)

Species and classification	Maximum measurement (cm[12])	Type of colour and pattern variation	Probable degree of exposure
Solenidae[20]			
Ensis ensis (L.)	12.70	III	(iii)
Ensis arcuatus (Jeffreys)	15.24	III	(iii)
Ensis siliqua (L.)	20.32	III	(iii)
Solen marginatus Montagu	12.70	III	(iii)
Cultellidae			
Cultellus pellucidus (Pennant)	3.81	III	(iii)
Tellinacea			
Tellinidae			
Tellina squalida (Montagu)	4.44	I	(ii)
Tellina tenuis (da Costa)	1.90	I	(ii)
Tellina fabula (Gmelin)	1.90	III	(ii)
Tellina donacina L.	2.54	II	(ii)
Tellina pygmaea Lovén	0.95	I	(ii)
Tellina crassa Pennant	6.35	II	(ii)
Tellina balaustina L.	1.90	II	(ii)
Gastrana fragilis (L.)	4.44	IV	(ii)
Macoma balthica (L.)	2.54	I	(ii)
Donacidae			
Donax[21] *vittatus* (da Costa)	3.81	I	(ii)
Donax variegatus (Gmelin)	3.81	II	(ii)
Psammobiidae[22]			
Gari fervensis (Gmelin)[23]	5.08	I?	(ii)?
Gari depressa (Pennant)	6.35	I	(ii)?
Gari tellinella (Lam)[23]	2.54	I	(ii)
Gari costulata (Turton)	2.54	I	(ii)
Scrobiculariidae			
Scrobicularia plana (da Costa)	6.35	IV	(iii)
Semelidae			
Abra tenuis (Montagu)	1.27	IV	(iii)
Abra alba (Wood)	2.54	IV	(iii)?
Abra nitida (Müller)	1.27	IV	(iii)?
Abra longicallus (Scacchi)	1.90	IV	(iii)?
Abra prismatica (Montagu)	2.54	IV	(iii)?
Solecurtidae			
Solecurtus scopula (Turton)	6.35	III	(iii)
Solecurtus chamasolen (da Costa)	6.35	III	(iii)
Pharus legumen (L.)	12.70	III	(iii)
(Dreissenacea, freshwater in Britain)			
(Gaimardiacea)			
Arcticacea			
Arcticidae			
Arctica islandica (L.)	12.70	II	(ii)
Glossacea			
Glossidae			
Glossus humanus (L.)	10.16	II	(ii)
(Corbiculacea, freshwater in Britain)			
Veneracea[24]			
Veneridae			
Dosinia exoleta (L.)	5.71	II	(ii)
Dosinia lupinus (L.)	3.81	II	(ii)
Gafrarium minimum (Montagu)	1.59	I	(ii)
Callista chione (L.)	8.89	II	(ii)
Venus verrucosa (L.)	6.35	II	(ii)
Venus casina (L.)[4]	5.08	III	(ii)
Venus ovata Pennant	1.90	III	(ii)
Venus fasciata (da Costa)	2.54	I	(ii)

APPENDIX A (Continued)

Species and classification	Maximum measurement (cm[12])	Type of colour and pattern variation	Probable degree of exposure
Venus striatula (da Costa)	4.44	II	(ii)
Venus mercenaria L.	12.70	III	(ii)
Venerupis aurea (Gmelin)	4.44	III	(ii)
Venerupis rhomboides (Pennant)	6.35	III	(ii)
Venerupis pullastra (Montagu)	5.08	II	(ii)
Venerupis saxatilis (Fleuriau)	3.81	IV	(iii)
Venerupis decussata (L.)	7.62	III	(ii)
Notirus irus (L.)	2.54	IV	(iii)
Petricolidae			
Petricola pholadiformis (Lam.)	6.35	IV	(iii)
Mysia undata (Pennant)	3.81	IV	(ii)
Myoida			
Myina			
Myacea			
Myidae			
Mya truncata L.	7.62	IV	(iii)
Mya arenaria L.	15.24	IV	(iii)
Sphenia binghami Turton[25]	1.27	IV	(iii)
Corbulidae			
Corbula gibba (Olivi)[4,26]	1.27	III	(ii)
Gastrochaenacea			
Gastrochaenidae			
Gastrochaena dubia (Pennant)	2.54	IV	(iii)
Hiatellacea			
Hiatellidae			
Hiatella arctica (L.)	3.81	IV	(iii)
Panomya arctica (Lam.)	7.62	IV	(iii)
Saxicava jeffreysi Winckworth	0.95	IV	(iii)
Pholadina			
Pholadacea			
Pholadidae			
Pholas dactylus L.	15.24	IV	(iii)
Barnea candida (L.)	6.35	IV	(iii)
Barnea parva (Pennant)	3.81	IV	(iii)
Zirfaea crispata (L.)	8.89	IV	(iii)
Pholadidea loscombiana Turton	3.81	IV	(iii)
Martesia striata (L.)	5.08	IV	(iii)
Xylophaga praestans Smith	1.90	IV	(iii)
Xylophaga dorsalis Turton	1.27	IV	(iii)
Teredinidae			
Teredo navalis L.	0.95	IV	(iii)
Lyrodus pedicellatus (Quatrefages)	0.63	IV	(iii)
Nototeredo norvagicus (Spengler)	1.90	IV	(iii)
Psiloteredo megotara (Forbes & Hanley)	1.27	IV	(iii)
Teredora malleolus (Turton)	1.27	IV	(iii)
Bankia fimbriatula Moll & Roch	0.63	IV	(iii)
(Hippuritoida†)			
ANOMALODESMATA			
Pholadomyoida			
(Pholadomyacea)			
Pandoracea			
Pandoridae			
Pandora[27] *albida* (Röding)	3.81	III	(ii)
Pandora pinna (Montagu)	1.90	III	(ii)
Lyonsiidae			
Lyonsia norwegica (Gmelin)[28]	3.81	IV	(ii)
Periplomatidae			

APPENDIX A (Continued)

Species and classification	Maximum measurement (cm[12])	Type of colour and pattern variation	Probable degree of exposure
Cochlodesma praetenue (Pulteney)[29]	3.81	IV	(iii)
Thraciidae			
Thracia[30] *phaseolina* (Lamarck)	3.81	IV	(iii)
Thracia villosiuscula (Macgillivray)	2.54	IV	(iii)
Thracia pubescens (Montagu)	8.89	IV	(iii)
Thracia convexa (Wood)	6.35	IV	(iii)
Thracia distorta (Montagu)	2.54	IV	(iii)
Poromyacea			
Poromyidae			
Poromya granulata (Westerdorp)	1.27	III	(ii)
Cuspidariidae			
Cuspidaria cuspidata (Olivi)	1.90	III	(ii)
Cuspidaria rostrata (Spengler)	2.54	IV	(ii)
Cuspidaria costellata (Deshayes)	0.95	IV	(ii)
Cuspidaria abbreviata (Forbes)	0.95	IV	(ii)
(Clavagellacea)			

[1]Extinct or unrepresented subclasses and orders, but not superfamilies, are shown for completeness. Only families represented in the British fauna are included. The order down to families is that of the *Treatise on Invertebrate Paleontology,* that of genera and species, and the binominal nomenclature (except as noted otherwise) is that of Tebble (1966). Introduced species, which are very few, have been included.

Orders etc. in parentheses are extant but not represented; extinct ones are marked with a †; for others that are omitted, the reason is given.

[2]Allen (1954a, 1960).

[3]m indicates the minute species disregarded in estimating the effect of size on colour and pattern.

[4]Allen (1960), deposition of manganese compounds on shell.

[5]The genetics of shell colour in *Mytilus edulis,* and the adaptive significance of its clinal variation with climate (east coast of North America) have been investigated by Newkirk (1980) and Mitton (1977) respectively. From the numerous studies on *M. edulis* L. and *M. galloprovincialis* Lamarck cited and added to by Gosling (1984) it appears best to treat the latter as a subspecies of *edulis.*

[6]Swimming, Baird (1958), Yonge (1949).

[7]Usually, since it becomes fixed like an oyster, considered as a separate genus, *Hinnites,* e.g. Abbott (1974).

[8]Seed & Roberts (1976) cast some doubt on the distinctness of *M. patelliformis* and *M. squama.* Material from Strangford Lough (seen by courtesy of Dr. Roberts) and material of *M. squama* identified by Winckworth (BM(NH)), seen by courtesy of Dr. P. Mordan) suggests, but does not prove, their distinctness.

[9]Defensive adaptations, Gilmour (1963, 1967).

[10]For discussions of preferred habitats (and habits) of Lucinacea, British and foreign, see Allen (1958a), Jackson (1973) and Kauffmann (1969).

[11]Diplodontidae in Tebble (1966).

[12]Morton, J. E. (1954).

[13]Morton, J. E. (1962).

[14]Said to be a neotenous veneracean by Ockelman (1964).

[15]Leaping, Ansell (1967a).

[16]Habits of *P. exiguum,* Russell & Petersen (1973).

[17]As *C. lamarcki* (Reeve) in Tebble (1966). Russell (1971) corrects the distribution in Tebble of *edule* and *glaucum* and (1972) reports character displacement in rib number between these two species.

[18]Leaping, Ansell (1969).

[19]Yonge & Allen (1985).

[20]Leaping, Ansell (1968).

[21]Biology of the genus, Ansell (1983).

[22]Gariidae in Tebble (1966).

[23]Leaping, Ansell (1967c).

[24]Habits, Ansell (1961).

[25]Habits, Yonge (1951).

[26]Habits, Yonge (1946).

[27]Habits, Allen (1954b), Allen & Allen (1955).

[28]Habits, Ansell (1967b).

[29]Habits, Allen (1958b). In Laternulidae, Tebble (1966).

[30]Habits, Yonge (1937).

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An asterisk (*) denotes a new taxon.

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