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MORFOLOGÍA DEL ESTOMAGO Y PARTES BLANDAS EN *MYTELLA STRIGATA* (HANLEY, 1843) (BIVALVIA: MYTILIDAE)

María Villarroel[1] y José Stuardo[2]

ABSTRACT

The anatomy of *Mytella strigata,* a species found in lagoons on the Pacific coast of central Mexico, is compared with that of the *M. charruana* of the Atlantic and of other marine Mytilidae, giving particular emphasis to the morphology of the stomach. The siphons belong to type A (Yonge, 1948), the ctenidia to type B(1) (Atkins, 1937), and the stomach to type III (Purchon, 1957) with sorting mechanisms of type B (Reid, 1965). Possible relationships between morphological adaptations and life habits are discussed.

Key words: stomach morfology, Bivalvia, Mytilidae, *Mytella strigata,* anatomy.

INTRODUCCIÓN

Muchos de los 23 géneros de la familia Mytilidae (Soot-Ryen, 1955) han sido estudiados desde el punto de vista anatómico funcional, entre ellos, *Mytilus* (White, 1937; Owen, 1974), *Botula* y *Lithophaga* (Yonge, 1955), *Musculus* (Merril & Turner, 1963), *Xenostrobus* (Wilson, 1967), *Adula* (*Botula*) (Fankboner, 1971), *Limnoperna* (Morton, 1973), *Modiolus* (Pierce, 1973; Morton, 1977), *Musculista* (Morton, 1974), y *Brachidontes* (Paiva Avelar & Narchi, 1984a, b).

El género *Mytella* está representado en la Provincia Panámica por cuatro o cinco especies, de las cuales cuatro se registran corrientemente en territorio mexicano: *Mytella guyanensis* (Lamarck, 1819), *M. strigata* (Hanley, 1843), *M. speciosa* (Reeve, 1857), y *M. tumbezensis* (Pilsbry & Olsson, 1935) (Keen 1971; García-Cubas & Reguero, 1987); aunque Bernard (1983) considera a las dos últimas como sinónimos.

La distribución de *M. strigata* en el Pacífico se extiende desde Guaymas, Sonora, México, hasta el sur de El Salvador e Islas Galápagos; pero, ocurre también en el Atlántico desde Venezuela hasta Argentina (Keen, 1971). En México, *M. strigata* se encuentra en abundancia en las lagunas costeras de Agiabampo, Topolobampo, Yavaros y Huizache-Caimanero de la costa del Golfo de California (García-Cubas & Reguero, 1987) y en la costa del Pacífico, en las lagunas de Nuxco y Chautengo del estado de Guerrero (Stuardo & Villarroel, 1976; Villarroel, 1978); Laguna de Cuyutlán y Bahía de Manzanillo (Colima) (Cobo et al., 1978); y en la costa de Oaxaca (Holguín & González, 1989).

Algunas especies del género *Mytella* han sido estudiadas en consideración a su importancia ecofisiológica y a su potencialidad como fuente de alimento. De *M. guyanensis* se tiene información acerca de su tamaño y concentración de metales (De Lacerda & Lima, 1983; De Lacerda et al., 1983), madurez sexual (Sibaja, 1986); sobrevivencia y capacidad de aislamiento en diferentes salinidades (Leonel & Silva, 1988).

Mytella strigata ha sido estudiada desde el punto de vista de su biología, ecología, prácticas experimentales de cultivo y morfometría (Stuardo & Rivera, 1976; Estévez, 1975; Stuardo & Estévez, 1977; Sibaja, 1985), a su contenido de glucógeno y grasa (Reprieto & Stuardo, 1975) y de metales pesados (Paez-Osuna et al., 1988), pero se conocen sólo observaciones generales sobre sus partes blandas. Sin embargo, un detallado estudio anatómico-funcional sobre *M. charruana,* realizado por Narchi & Galvão-Bueno (1983), nos permite comparar sus resultados con los obtenidos en *M. strigata* y extrapolarlos a nivel genérico.

MATERIALES Y MÉTODOS

Se obtuvieron 200 ejemplares de diferentes edades de las lagunas de Nuxco (100°47'N, 100°49'W) y Chautengo (99°02'N, 99°09'W) (Guerrero; Nov. 1974–Mayo 1975) y

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de la Laguna de Cuyutlán, (19°02'N, 104°19'W) (Colima; Ago. 1977–Jul. 1978). La colecta se realizó de forma manual desprendiendo del lodo los grupos de ejemplares que yacen sobre el fondo.

Las disecciones anatómicas se realizaron en ejemplares adultos de 50 a 70 mm de longitud, previamente fijados en formol al 5% y preservados en alcohol etílico al 70%, utilizando un microscopio estereoscópico Zeiss. Para precisar los detalles de poco contraste se utilizó el colorante rojo neutro.

Las figuras de las estructuras internas se hicieron directamente al microscopio utilizando ejemplares fijados, y ejemplares vivos para el borde del manto. En la descripción de la musculatura, se usó la terminología de Graham (1934a, b) y en la del estómago las de Graham (1949) con las modificaciones sugeridas por Owen (1953) y Purchon (1957, 1958, 1960) y en especial por Dinamani (1967). La Figura 3 que muestra el interior del estómago, se hizo después de realizar un corte en la pared dorsal desde el esófago hasta el saco del estilo. Los términos derecho e izquierdo, aplicado a las estructuras estomacales, se refieren a esta línea media.

RESULTADOS Y DISCUSIÓN

Concha.

La concha de *M. strigata* es mitiliforme, generalmente algo cóncava en su parte ventral, y de forma aguzada ó más ó menos ensanchada anteriormente. Los umbos son subterminales a casi terminales. El margen dorsal es regularmente curvado. En la cara externa, se observan a menudo estrías radiales finamente marcadas en el tercio anterior y escasas, pero más marcadas, en la mitad posterior, sobre todo internamente. El perióstraco es brillante, y su coloración variable entre amarillo verdoso claro a casi negro, uniforme o sombreado de verde o pardo amarillento en los márgenes anterior, dorsal posterior y especialmente el ventral. Preferentemente en los ejemplares juveniles o medianos, se observan bandas oscuras radiales o entrecruzadas que resaltan sobre una superficie más clara o como manchas zigzageantes o jaspeadas de color pardo.

En la cara interna se observan de 2 a 4 pliegues radiales, a manera de dientes en el margen anterior. El ligamento es muy alargado llegando hasta la mitad de la concha. La impresión del aductor anterior es relativamente grande y la del aductor posterior es grande y redondeada; sobre esta última y hacia adelante de ella, se encuentra la huella del retractor posterior (Fig. 1a). La coloración interna es violácea oscura, brillante.

La longitud máxima de la concha constatada por nosotros en esta especie es de 80 mm, aunque tales tamaños corresponden aparentemente sólo a unos pocos individuos en cada población. Sibaja (1985), en un estudio del crecimiento de la concha en una población de *M. strigata* de la Playa de Lepanto, Puntarenas, Costa Rica, encontró que es alométrico y que el largo es un parámetro adecuado para evaluar crecimiento. Sin embargo, la concha crece más en altura y es comparativamente más esférica que en *M. guyanensis.* Las medidas mínimas y máximas obtenidas por este autor fueron de 10 y 42.6 mm y los promedios calculados iguales a 24.9, 10.8 y 7.8 mm para el largo, ancho y alto, respectivamente. Estas medidas de longitud máxima son inferiores a las constatadas por Estévez & Stuardo (1977) en poblaciones de la costa Pacífica mexicana, en donde observaron máximos de hasta 70 mm, con promedios de hasta 49.6 mm en diversas lagunas.

Manto y Aberturas Sifonales.

Los lóbulos del manto en *M. strigata* se encuentran unidos en la región dorsal, en toda su longitud, desde el extremo anterior hasta la parte dorsal del área anterior del músculo retractor pedal posterior (Fig. 1a), a diferencia de otros mitílidos (e.g. *Mytilus edulis*) en donde la unión llega hasta la mitad de la región del aductor posterior (Bullough, 1958). En cambio, en *Mytella charruana, Brachidontes darwinianus* y *B. solisianus* esta unión llega hasta la región posterior al aductor posterior (Narchi & Galvão-Bueno, 1983; Paiva Avelar & Narchi, 1984a, b).

El borde del manto está compuesto de un delgado pliegue externo, un pliegue medio, moderadamente delgado y de un pliegue interno relativamente grueso, con su borde enrollado y con tentáculos cortos o papilas digitiformes. En *M. charruana* el borde interno es liso, sin fusiones y totalmente libre; además, los bordes medios y externo son contínuos, musculares y unidos en la región posterior para formar un sifón (Narchi & Galvão-Bueno, 1983). Según White (1937), en *Mytilus edulis* este pliegue es ciliado y muy sensible al tacto

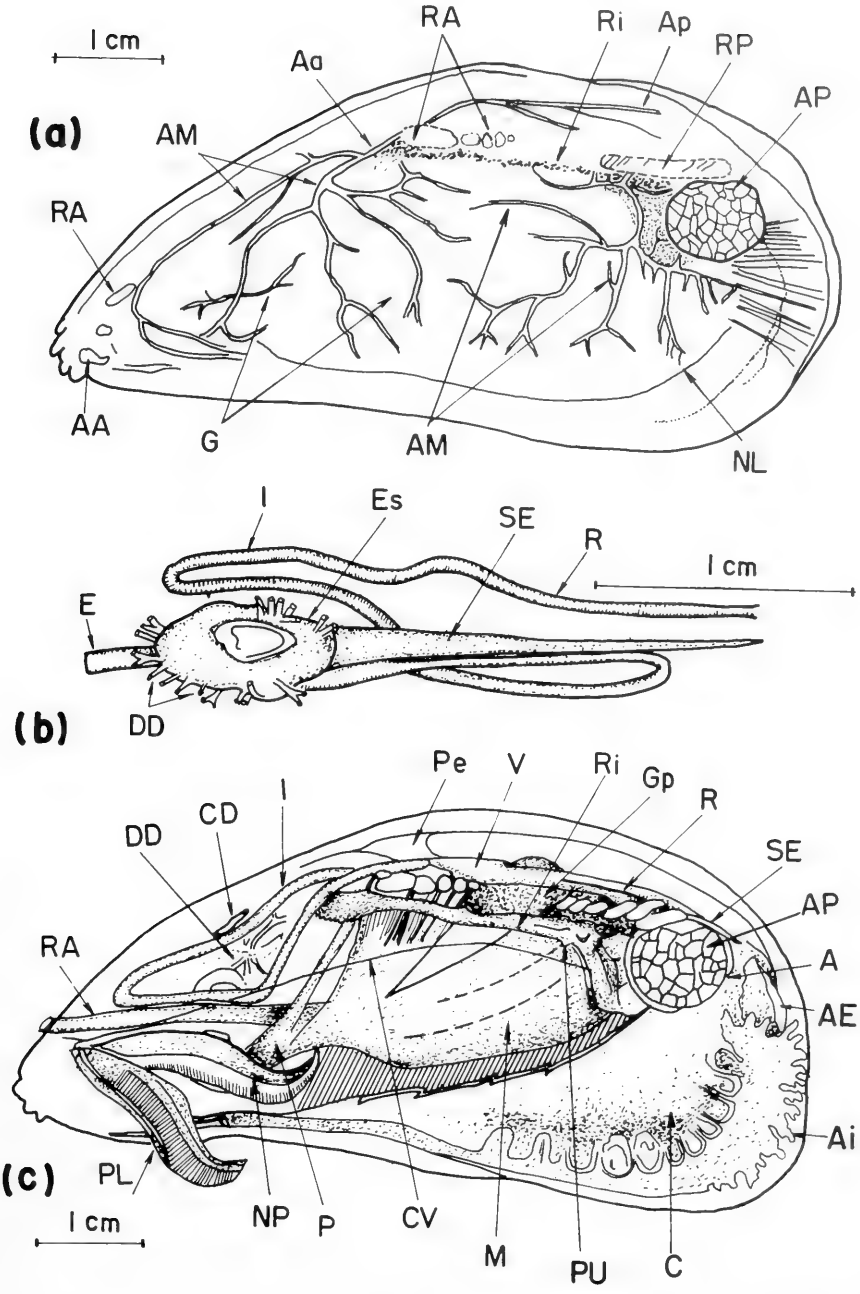

FIG. 1. *Mytella strigata.* (a) Manto izquierdo. (b) Vista ventral del tracto digestivo. (c) Cavidad paleal, sin branquia izquierda ni glándula digestiva.
A, ano; AA, aductor anterior; Aa, aorta anterior; AE, abertura exhalante; Ai, abertura inhalante; AM, arteria del manto; AP, aductor posterior; Ap, aorta posterior; C, ctenidio; CD, capuchón dorsal; CV, conectivo visceral; DD, ductos de los divertículos digestivos; E, esófago; Es, estómago; G, gónada; Gp, glándula pericárdica; I, intestino; M, mesosoma; NL, nervio paleal; NP, nervio del palpo; P, pie; Pe, pericardio; PL, palpo labial; PU, papila urogenital; R, recto; RA, retractor pedal anterior; Ri, riñón; RP, retractor pedal posterior; SE, saco del estilo; V, ventrículo.

y a repentinos cambios de intensidad luminosa.

Aberturas Exhalante e Inhalante.

El pliegue interno de los lóbulos del manto se une posteriormente, en su tercio superior, para formar la abertura exhalante con los márgenes lisos y protruidos debilmente. Así mismo, esta abertura queda limitada ventralmente por la parte terminal de la membrana branquial, que forma un septo triangular. La unión continúa por un trecho corto, pero el resto del borde queda libre, dejando una gran abertura ventral que corresponde a la abertura inhalante, con papilas ó tentáculos digitiformes sólo en su región posterior, de manera similar a lo observado en *M. charruana* (Narchi & Galvão-Bueno, 1983). Yonge (1948) clasifica a este tipo de aberturas como sifones del tipo A, aunque no las considera sifones verdaderos.

No es posible establecer diferencias entre las especies de *Mytella* utilizando las caracteristicas de las papilas de los bordes del manto, como lo sugiriera Soot-Ryen (1955). Debido a su gran variación y, en consecuencia, al menos en este caso, parecen no representar un carácter morfológico de valor taxonómico. Las digitaciones o tentáculos del manto se observaron de color blanco y poco arborescentes en ejemplares pequeños (juveniles); en cambio, en ejemplares grandes (maduros), tanto los márgenes del manto, las digitaciones y el septo presentaron manchas pardas oscuras y las digitaciones más arborescentes.

Al igual que en otros mitílidos, en época de maduración sexual, cada lóbulo del manto se observa engrosado por las gónadas que se extienden en ellos.

Músculos Aductores y del Pie.

El aductor posterior en *Mytella strigata* tiene una posición semejante a los de *M. charruana* y de *Mytilus edulis.* Sin embargo, entre estos dos géneros se puede apreciar una diferenciación en lo que respecta a los retractores del pie. En *Mytella,* la rama posterior del retractor posterior alcanza hasta la mitad del aductor posterior (Fig. 1a, c, RP, AP); en cambio, en *Mytilus* el retractor posterior queda sólo adosado al aductor posterior. Además, en *Mytella* se distinguen dos paquetes de retractores pedales; ésto es, los anteriores están separados de los posteriores (Fig. 1a, c, RA, RP), como en *Choromytilus,* en cambio en *Mytilus* los haces están contíguos. Las diferencias funcionales no se han determinado pero, aparentemente están ligadas a una mayor actividad del animal.

Pie.

El pie de *M. strigata* (Fig. 1c, p), como en otros mitílidos, es pequeño, de color pardo obscuro y con un profundo surco posterior. No hay una variación apreciable en la forma del pie respecto de las otras especies conocidas.

Ctenidios.

En *M. strigata,* al igual que en *M. charruana,* la demibranquia interna es un poco menor que la externa. De acuerdo a Atkins (1937) pertenece al tipo de ctenidio B(1) característico de Mytilidae y Pinnidae. No hay extensión supraxial (Fig. 1b, c, que muestra parte de las lamelas del lado derecho). Son branquias planas y homorrábdicas con una distribución de tractos ciliares descritos en detalle en *M. charruana* por Narchi & Galvão-Bueno (1983).

Palpos Labiales.

Los palpos de *M. strigata,* como los de *M. charruana* (Narchi & Galvão-Bueno, 1983) y *Modiolus metcalfei* (Morton, 1977), son muy grandes y alargados, en comparación con los de las especies de *Mytilus* en que son casi triangulares. Su longitud es tal, que alcanza hasta el pie y la parte anterior del mesosoma (Fig. 1c, PL, M); hay numerosos surcos que se aprecian por transparencia (Fig. 1c). Entre las láminas externa e interna de los palpos derechos e izquierdos, hay un surco ciliado profundo, que conduce a la boca.

Tubo Digestivo.

El curso y las características del aparato digestivo en *M. strigata* (Fig. 1b, c) son, en general, similares a los de *M. charruana,* excepto el largo y posición de la vuelta anterior del intestino, que en *M. strigata* es lateral izquierda, larga, y en *M. charruana* es más dorsal y más corta. En ambas especies el saco del estilo y el intestino salen en forma paralela separadamente hacia atrás, terminando sobre el tercio anterior del aductor posterior en *M. charruana,* y sobre el ano,

más atrás del aductor posterior en *M. strigata.*

Con respecto a *Mytilus edulis* y las especies de *Xenostrobus* hay una notable diferencia en el curso de la vuelta anterior del intestino, ya que en ellas, el intestino al volver desde atrás, después de separarse del saco del estilo, pasa de dorsal derecho a ventral al esófago y se vuelve a dirigir hacia atrás por el lado izquierdo (Bullough, 1958: fig. 141; Wilson, 1967: fig. 3). En los otros géneros que han sido estudiados por diversos autores, que se mencionan en el siguiente párrafo, no se ha descrito el curso del tubo digestivo.

Estómago.

Estómagos de varios géneros de Mytilidae han sido descritos anteriormente en detalle. Así ocurre para tres especies de *Modiolus: M. modiolus* (Nelson, 1918; Reid, 1965), *M. undulatus* y *M. striatulus* (Dinamani, 1967). Dos especies de *Lithophaga: L. nasuta* (Purchon, 1957) y *L. gracilis* (Dinamani, 1967); *Limnoperna fortunei* (Morton, 1973); *Musculista senhausia* y *Modiolus melcalfei* (Morton, 1977); *Adula (Botula) falcata* (Frankboner, 1971); *Mytilus edulis* (Graham, 1949; Reid, 1965); *Perna viridis, Arcuatula* sp. y *Botula cinnamomea* (Dinamani, 1967); *Mytella charruana* (Narchi & Galvão-Bueno, 1983); y *Brachidontes solisianus* y *B. darwinianus darwinianus* (Paiva Avelar & Narchi, 1984a, b)

La nomenclatura adoptada en la siguiente descripción, es la propuesta por Owen (1953).

El estómago de *M. strigata* (Fig. 2d, e), al igual que en *M. charruana,* es largo y aplastado dorsoventralmente, diferente del de *Mytilus edulis* que es corto. En su estructura interna se asemeja más al estómago de *Perna viridis,* descrito por Dinamani (1967).

Como se ilustra en las Figuras 2b y 3, el esófago (E) desemboca anteriormente (E′) y presenta repliegues longitudinales internos (RF) que terminan en una elevación que circunda su entrada al estómago; un surco longitudinal corre a cada lado de esta elevación. Por ambos lados del estómago (Figs. 1b, 2a, c-e) entran numerosísimos ductos (más o menos 34) provenientes de los divertículos digestivos (DD) y posteriormente, salen juntos el saco del estilo (SE) y el intestino (I).

Externamente y en vista dorsal (Fig. 2c) son también conspicuos: el extremo anterior del ciego seleccionador de alimento (CSD); hacia el lado izquierdo el capuchón dorsal (CD); debajo y hacia atrás, la bolsa izquierda (BI); en la linea media un bolsillo en el que se transparentan surcos (Ca) y hacia la izquierda del mismo, el área de selección posterior (APS).

Ventralmente (Fig. 2a), la estructura más notable es el ciego seleccionador de alimento (CS), aplanado y casi tan largo como el estómago mismo, en el que se transparenta el curso del tiflosol mayor y el surco intestinal. A la derecha de este último, y bajo el capuchón dorsal, nace el intestino (I) junto al saco del estilo, pero no dentro de él; ambos se encuentran estrechamente unidos.

Internamente, hay similitud con los rasgos generales descritos para otros géneros, pero se constatan diferencias en el ciego seleccionador de alimento, la distribución de los ductos de los divertículos digestivos y la configuración del repliegue axial, como se describe a continuación.

En *M. strigata* el tiflosol mayor (Fig. 3, TY) emerge del intestino (I) y corre hacia adelante por el lado derecho sobre el piso del estómago, se curva hacia la izquierda y entra al ciego seleccionador de alimento (CS). Dentro de este ciego (Fig. 2b) da una vuelta completa en espiral en dos planos; luego sigue hacia atrás hasta llegar casi al extremo del ciego, se curva hacia arriba manteniendo el mismo plano y, nuevamente, en sentido inverso, sigue hasta el fondo del saco y se vuelve a curvar hacia arriba. Manteniendo el mismo plano anterior llega a la mitad del ciego, y siguiendo una amplia curvatura toma el sentido espiral de la primera vuelta y abandona el ciego por encima de donde entró. En total da cuatro vueltas, dos en cada sentido.

En *Modiolus modiolus,* al igual que en *Mytilus edulis,* el tiflosol mayor presenta una sola vuelta; sin embargo, el ciego es más corto en *Modiolus* (Nelson, 1918; Reid, 1965: fig. 6). En *Modiolus undulatus* el tiflosol mayor entra en un plano y regresa en otro sobre sí mismo, ésto es, da una sola vuelta en dos planos; en cambio en *Arcuatula* sp. también experimenta una vuelta, pero en el mismo plano. En *Mytilus striatulus* el ciego está poco desarrollado, apenas como una depresión. El tiflosol muestra un pequeño embahiamiento y termina más arriba en la pared izquierda. Situación semejante, aunque más sinuosa, se presenta en *Lithophaga gracilis* y en *Botula cinnamomea* (Dinamani, 1967).

El término del tiflosol mayor (TY′), en *M. strigata* y *Perna viridis* se presenta una vez

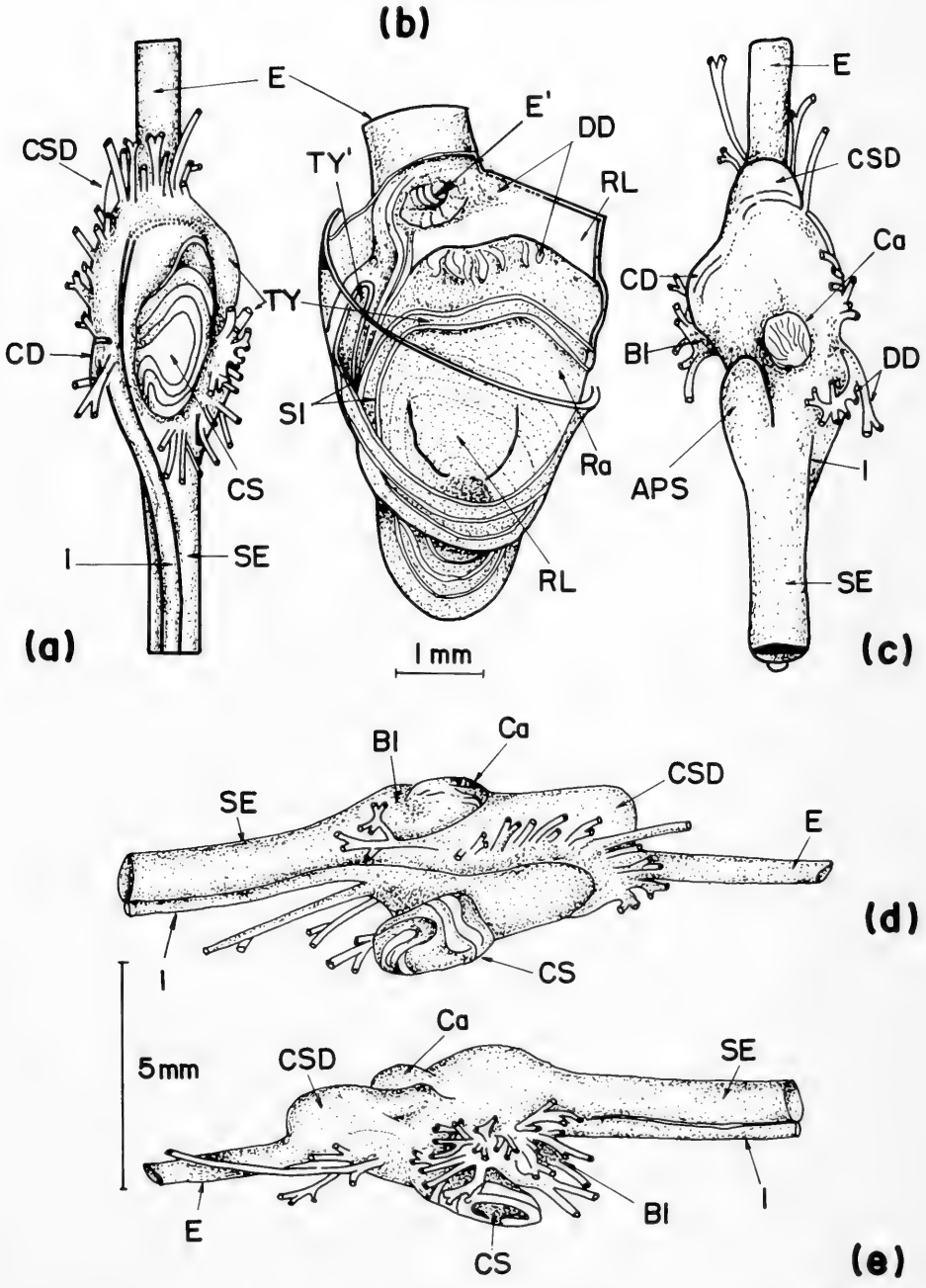

FIG. 2. *Mytella strigata.* Estómago. (a) Vista ventral. (b) Detalle del ciego seleccionador de alimento. (c), (d) y (e), Vistas dorsal, lateral derecha e izquierda respectivamente. (a), (c), (d) y (e) están en la misma escala. APS, Area de sección posterior; BI, bolsa izquierda; Ca, ciego del APS; CD, capuchón dorsal; CS, ciego seleccionador; CSD, prolongación dorsal del CS; DD, ductos de los divertículos digestivos; E, esófago; E′, abertura esofágica; I, intestino; Ra, repliegue axial; RL, repliegue lateral; SE, saco del estilo; SI, surco intestinal; TY, tiflosol mayor; TY′, término TY.

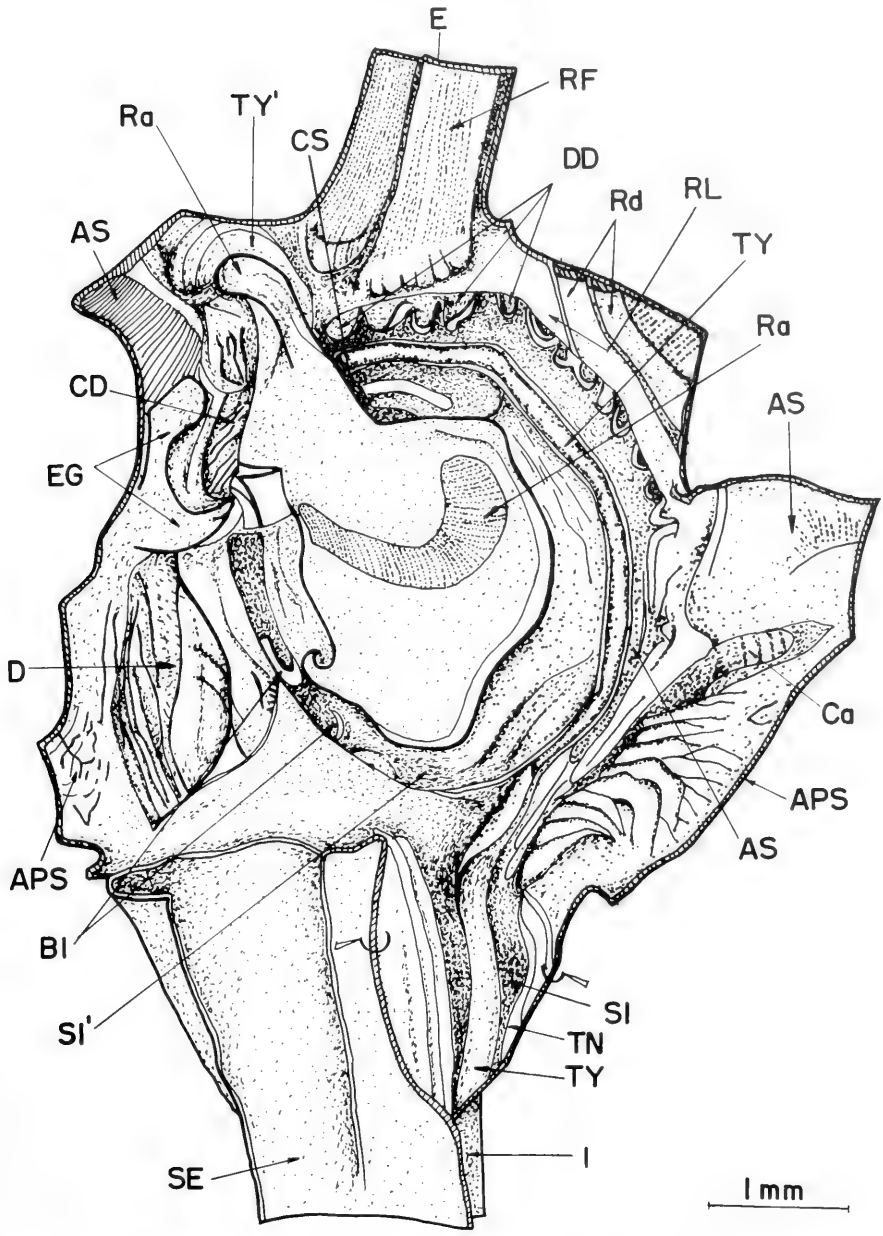

FIG. 3. Interior del estómago de *Mytella strigata* abierto por un corte longitudinal dorsal.
AS, área de selección; APS, AS posterior; BI, bolsa izquierda; Ca, ciego del APS; CD, capuchón dorsal; CS, ciego seleccionador; D, dientes del EG; DD, ductos divertículos digestivos; E, esófago; EG, escudo gástrico; I, intestino; Ra, repliegue axial; Rd, repliegues dorsales; RF, repliegues esofágicos; RL, repliegue lateral; SE, saco del estilo; SI, surco intestinal; SI′, comienzo del SI; TN, tiflosol menor; TY, tiflosol mayor; TY′, término del TY.

que el tiflosol emerge del ciego seleccionador de alimento, sobre la pared izquierda lateral a la abertura esofágica. En cambio, en *Modiolus modiolus* (Nelson, 1918) y en *Mytilus edulis* (Graham, 1949) termina dentro del ciego seleccionador de alimento.

Todos los ductos de los divertículos digestivos del lado derecho (18), están distribuídos en una línea a intervalos uniformes, y desembocan en una "depresión" a la derecha del tiflosol mayor, que se comunica anteriormente con el ciego seleccionador de alimento y, posteriormente, conduce hacia el área posterior de selección. Dicha distribución es semejante a la de *Botula cinnamomea* y más o menos similar en *Mytilus edulis,* pero distinta a la de *Lithophaga gracilis,* en la que los ductos están ordenados en tres grupos (Dinamani, 1967: fig. 8).

A la derecha y arriba de esta "depresión," hay dos repliegues dorsales (Fig. 3, Rd), con un surco profundo entre ellos, que comienza como un pequeño reborde; los repliegues salen desde la plataforma anterior al ciego del área posterior de selección y terminan en la entrada del capuchón dorsal. Las dimensiones en su origen son mayores que el tiflosol mayor y van disminuyendo gradualmente hasta casi desaparecer en su término.

En la parte dorsal del estómago, a la derecha de los repliegues antes descritos, se encuentra un área de selección (AS) con numerosos pliegues y surcos finos y uniformes que corresponden al tracto anterodorsal de Reid (1965). Este tracto relaciona, en parte, al capuchón dorsal con el ciego del área posterior de selección. Su unión directa está impedida por un área lisa junto al ciego del área posterior de selección y por una prolongación del escudo gástrico en la entrada del capuchón dorsal.

El capuchón dorsal (CD), a diferencia de las otras especies en que se ha descrito, presenta un área de pliegues y surcos que sin duda corresponde a un área de selección. Su presencia en esta estructura (considerada sólo de almacenamiento), junto con los otros caracteres ya mencionados, implica una mayor adaptación a la selección de partículas.

Los ductos de los divertículos digestivos del lado izquierdo se abren tanto en el área deprimida que se encuentra entre el escudo gástrico y el repliegue axial, a la entrada de la bolsa izquierda (BI), como dentro de la misma.

El conjunto del ciego seleccionador de alimento con los ductos de los divertículos digestivos, la continuación del tiflosol mayor y el surco intestinal, representan el mecanismo principal de selección del estómago y corresponde al tipo B de Reid (1965).

El surco intestinal (SI) se origina cerca de la bolsa izquierda (SI'), sigue a la izquierda del tiflosol mayor en todo su curso por el piso del estómago, entra luego al ciego seleccionador de alimento y regresa por la derecha del tiflosol hacia el intestino.

Un repliegue aplanado (RL), denominado repliegue lateral por Dinamani (1967) en *Perna viridis* y descrito anteriormente por Graham (1949) como "fold" en *Mytilus edulis,* comienza donde termina el tiflosol mayor. Este repliegue pasa por las paredes del ciego seleccionador; al salir, gira hacia la derecha y sigue bajo el esófago formando una plataforma; se vuelve luego hacia atrás y corre en dirección paralela al tiflosol mayor sobre el lado derecho del estómago. En el hecho, constituye una pared delante del área de selección posterior, y deja entre él y el surco intestinal una pequeña área plegada que, quizás, represente un área menor de selección.

En *M. strigata,* el área posterior de selección, ocupa una especie de bolsillo sobre la pared dorsal izquierda (Fig. 3, APS), con un gran desarrollo de repliegues y surcos, en comparación a las otras especies. Efectivamente, en las especies de *Modiolus* descritas por Dinamani (1967) no hay área posterior de seleción, sino que aparenta haber pequeños abultamientos sobre la pared derecha. En *M. undulatus* tiene la forma de una depresión baja; en *M. striatulus* es más pronunciada y en *Arcuatula* existe un área a manera de canal que se extiende desde el intestino, donde va también el tiflosol menor.

El ciego del área posterior de selección (Ca), que de acuerdo a Reid (1965) no es funcional en *Mytilus edulis,* está bien desarrollado en *M. strigata,* presentando pliegues y surcos bien notorios; éstos desempeñan probablemente una función seleccionadora. Además, el ciego está casi separado del área principal, situación que tampoco presenta *Perna viridis.*

El área posterior de selección está limitada ventral y anteriormente por un surco profundo, que corresponde al surco de rechazo (SR) descrito para algunos eulamelibranquios (Owen, 1953; Dinamani, 1967); su función es la de drenar el área de selección posterior y llevar las partículas al surco intestinal.

Otra zona del estómago que presenta un área de pliegues muy finos, comparable solo a la descrita por Dinamani (1967: fig. 4a) en *Perna viridis,* se encuentra sobre el pliegue axial (Ra). Este repliegue axial ocupa casi todo el piso del estómago desde el ciego se-

leccionador de alimento hasta la bolsa izquierda y es tan prominente como en las especies de *Modiolus.*

El escudo gástrico (EG) no es muy grande. Presenta dos prolongaciones anteriores hacia el capuchón dorsal; una muestra su extremo romo y la otra un par de fuertes dientes. Detrás de estos últimos, existe un área deprimida con varias líneas longitudinales de dientes quitinosos muy pequeños. Este carácter, que indudablemente ayuda en la mejor desintegración de las partículas alimenticias, no ha sido descrito en otros mitílidos.

Recto.

El recto, después de dejar la cavidad pericárdica y atravesar al ventrículo, desciende por la parte posterior del aductor posterior y desemboca en el ano (A).

Es difícil adjudicar alguna ventaja funcional al plan del recto atravesando la cavidad pericárdica de estos mejillones y sus modificaciones o derivar este ordenamiento de alguna explicación embriológica o fisiológica. A este respecto, en *M. strigata* se observa otra diferencia con *Mytilus edulis,* ya que el recto luego de abandonar el ventrículo y cavidad pericárdica pasa dorsalmente sobre el complejo de músculos comprendidos por el aductor posterior, el retractor posterior del biso y el retractor pedal posterior.

Por otra parte, Pierce (1973) encontró diferencias en la morfología interna del recto de los mitílidos estudiados por él. Comparando sus resultados con lo encontrado por nosotros en *M. strigata* el tiflosol del recto es algo similar al de *Modiolus demissus granosissimus,* aunque un poco más aplastado y completamente diferente al de *Mytilus edulis, Modiolus squamosus* e *Ischadium recurvum,* en los que el tiflosol no está bien definido.

Sistema Circulatorio.

Quitando las valvas de ejemplares donde la gónada se encuentra en estado de reposo o indiferenciado (Fig. 1a), se aprecian en primer plano las ramificaciones de la aorta anterior (Aa), la aorta posterior (Ap) y las arterias del manto anterior y posterior (AM).

La aorta anterior se bifurca delante del retractor anterior del pie, originando la arteria anterior del manto (AM) y otra rama que se dirige hacia las vísceras. Las ramificaciones de la arteria anterior del manto cubren la mitad anterior del mismo. La rama más anterior de esta arteria forma un circuito cerrado, como se aprecia en la Figura 1a. La aorta posterior sólo irriga la parte superior del manto. La arteria posterior del manto aparece por debajo de la parte anterior del retractor posterior del pie, y se ramifica repetidas veces cubriendo la mitad posterior del manto.

El corazón se encuentra dentro de la cavidad pericárdica (Fig. 1c), ubicada casi posterior a los músculos retractores pedales anteriores.

Las aurículas, cubiertas por la glándula pericárdica, están tan alargadas hacia atrás que llegan a alcanzar las terminaciones de los retractores posteriores del pie, sin curvarse luego hacia el lado contrario. La glándula pericárdica presenta un aspecto granular aceitoso.

La sangre entra a las aurículas lateralmente desde numerosos senos pequeños que están ligeramente tapizados y oscurecidos por los órganos de Keber, y abandona el ventrículo anteriormente por la aorta anterior, que desemboca en un bulbo aórtico que sale del pericardio.

El ventrículo (V) es alargado y está atravesado por el recto (R) en toda su longitud. Como en la mayoría de los mitílidos (Fig. 1c), el ventrículo está suspendido desde cuatro puntos: anteriormente de la aorta y el recto; posteriormente del recto y lateralmente de las aurículas. El recto pasa longitudinalmente a través de todo el lúmen del ventrículo y de ahí hacia atrás a través de todo el largo de la cavidad pericárdica. Tal secuencia de recto y ventrículo ha sido descrita en varios mitílidos (Field, 1922; White, 1942; Jegla & Greenberg, 1968) y en particular para *Modiolus squamosus* (Pierce, 1973). En cambio, la suspensión de los ventrículos de "*Modiolus*" *demissus* e *Ischadium recurvum* es completamente diferente del plan típico de los mitílidos, y resulta de un modelo modificado del paso del recto a través de la cavidad pericárdica. Efectivamente, el recto pasa solamente a través de la porción anterior del ventrículo y luego, emergiendo desde la superficie dorsal del ventrículo, se arquea dorsalmente en su propia envoltura a lo largo del techo de la cavidad pericárdica, para después subir en el extremo posterior de la cavidad. La mitad posterior del ventrículo, no soportada por el recto, cuelga libremente en la cavidad pericárdica, y su extremo anterior está suspendido y anclado por las aurículas y el recto. Según Pierce (1973), una consecuencia fisio-

lógica obvia de su ordenamiento es que la dirección del batir ventricular en estas dos especies es postero-anterior, más que lateral a medio como en la mayoría de los mitílidos.

Organos Excretores.

El riñón (Figs. 1a, c, Ri) en *M. strigata* se encuentra a ambos lados de la base de la branquia. Desde la parte anterior del retractor del pie, donde se ensancha un poco, pasa lateralmente por toda la masa visceral, conservando más o menos el mismo diámetro hasta el aductor posterior, donde se ensancha notablemente y desciende finalmente por debajo del mismo. Esta situación es diferente en *Mytilus edulis,* donde la parte anterior llega hasta la región de los palpos y la parte posterior hasta el límite posterior del aductor (Bullough, 1958: fig. 141) al igual que en *Brachidontes darwinianus* y *B. solisianus* (Avelar & Narchi, 1984 a, b).

El riñón, a ambos lados de la masa visceral, aparece como una bolsa pardo oscura con digitaciones notables hacia el extremo anterior y el posterior. Está unido por una angosta banda de tejido renal al organo de Keber o "glándula pericárdica" (Gp). Hay una abertura interna muy poco visible en el pericardio y una abertura externa de la papila urogenital (PU). A esta papila se abren uno al lado del otro el ducto renal y el gonoducto.

Sistema Nervioso

El sistema nervioso, en su aspecto general, no presenta variaciones notables en el análisis comparativo.

Los dos ganglios cerebrales se encuentran situados posteroventralmente a los bordes de la boca. Un conectivo cerebro-visceral los une al ganglio visceral y una bifurcación de éste (el conectivo cerebro-pedal), al ganglio pedal. Cada ganglio visceral se encuentra en posición antero-ventral al músculo aductor posterior en la línea de fijación de los ctenidios. Los dos ganglios pedales están estrechamente unidos en la parte más profunda del extremo proximal del pie.

El nervio más prominente del ganglio cerebral es el nervio anterior del manto. En el caso del ganglio pedal, es el nervio pedal el que pasa hacia abajo en el pie; en el del ganglio visceral es el nervio posterior del manto que corre por toda la periferia de éste.

Sistema Reproductor.

Las gónadas (Fig. 1a, G) se encuentran extendidas en los lóbulos derechos e izquierdo del manto y entre los órganos de la masa visceral. Las regiones ventrales de las gónadas derecha e izquierda llenan completamente el mesosoma (M).

Los gonoductos se abren a lo largo del ducto renal en la papila urogenital (PU).

CONCLUSIONES

Mytella strigata, al igual que *M. charruana* y *Modiolus demissus,* es una forma infaunal o semiinfaunal que se entierra en el sustrato blando para ganar estabilidad y protección, aunque el presentar biso, la capacita para fijarse a cualquier tipo de sustrato vecino duro o semiduro.

En las lagunas estudiadas se encontró viviendo en fondos areno-limosos con alto contenido de materia orgánica y restos de conchas, formando bancos de extensión y abundancia considerables (Stuardo & Villarroel, 1976; Stuardo & Estévez, 1977; Villarroel, 1978). En estos bancos, como consecuencia de la falta de sustrato duro, los ejemplares se fijan unos a otros, de modo que llegan a formar masas como "racimos" que se mantienen sobre el sustrato y parcialmente enterradas, debido a la tranquilidad de las aguas y a la falta de corrientes notorias. Sin embargo, cualquier tipo de sustrato duro (rocas, raíces de mangle, etc.) y por supuesto, sustratos artificiales, determinan la fijación muy numerosa de ejemplares. En la Laguna de Chautengo los bancos se encontraron concentrados en la mitad oriental y sobre todo en el sector noreste; en la laguna de Nuxco, cubrían gran parte de los fondos, salvo en las zonas más profundas, pero la mayor concentración correspondió a los sectores marginales y en particular, a la región oriental cercana a la barra y al canal, como lo ilustran los mapas de Stuardo & Estévez (1977).

En la Laguna de Cuyutlán existían bancos en zonas con características típicamente lagunares, pero al abrir artificialmente el Canal Ventanas, desaparecieron por el cambio hacia condiciones marinas.

Mytella como *Modiolus,* no anida en el lodo con el biso como ocurre en *Musculus, Musculista* y *Amyqdalum,* a los que Morton (1977) considera más especializados. Por lo tanto,

Mytella puede considerarse una forma intermedia entre los que anidan y los altamente especializados como *Mytilus, Septifer* y *Limnoperna* de la epifauna, como lo sugieren Narchi & Galvão-Bueno (1983).

Considerando distintas opiniones, los caracteres que refuerzan su estado infaunal o semiinfaunal son: condición anisomiaria extrema; concha de contorno triangular con umbos recurvados o con forma de gancho, que la capacita para anclarse mejor o adherirse a las superficies redondeadas de ejemplares de mejillones vecinos; palpos y branquias bien desarrollados, que junto a los grandes retractores posteriores favorecen la ventilación y eliminación de partículas de sedimento; y un estómago con complejos tractos de selección para la selección de partículas de alimento. Estas características definirían también una condición primitiva entre los Mytilidae (Yonge & Campbell, 1968).

Efectivamente, la reducción de la región anterior de la concha en los Mytilidae está acompañada por la disminución del tamaño del aductor anterior. Ya Yonge (1953) lo sugirió como una adaptación en estos organismos gregarios, para elevar la región posterior de la concha, de modo que no sean obstruídas las corrientes inhalantes de individuos muy cercanos. Una alternativa propuesta por Stasek (1966), sugiere que la reducción anterior puede haber evolucionado en los mitílidos de regiones tropicales, donde la productividad es baja, como una forma de aumentar la captación de alimento. Según Morton (1977), la condición heteromiaria es primitiva e indica que el hábito de enterrarse precede al epifaunal.

Por otra parte, Stanley (1970) relaciona el área de la corriente inhalante y el grado de bombeo con el tamaño y estructura de los órganos de bombeo (las branquias), los que podrían ser más importantes en este aspecto. Es probable que en *M. strigata* y *M. charruana* la disminución de la abertura de los bordes del manto ayude a impedir la entrada de fango a la abertura exhalante y a favorecer la tasa de bombeo. También es posible que la presencia de gran cantidad de papilas en el borde del manto, juegue un mayor papel en la detección de la calidad del agua circundante, y la filtración y rechazo de las partículas de limo-arcilla en aguas calmas, o de arena fina en hábitat rocosos con alta energia, como se ha observado en *Mytilus edulis* y en *Perna perna* (Narchi & Galvão-Bueno, 1983).

Los palpos de *M. strigata* y *M. charruana* presentan mayor tamaño y, por consiguiente, mayor número de surcos y pliegues que los de *Mytilus edulis,* lo que indica un mayor grado de selectividad de particulas alimenticias (materia orgánica particulada, fito y nanoplancton). Esta situación se corrobora también por la presencia de un gran número de filamentos branquiales.

Otros caracteres importantes que apoyan la adaptación de *Mytella* a los fondos blandos y explican su forma de alimentación son: el estómago de tipo III de Purchon (1957) con varias áreas de selección, y dentro del mismo, el rol del ciego seleccionador de alimento. Este actuaría más como un reservorio temporal de alimento que como un ciego de selección, capacitando a esta especie para períodos de ayuno largos, como lo trata de demostrar Dinamani (1967) con base en sus observaciones en *Perna viridis.* Pero, también parece plausible considerar a este ciego como una estructura seleccionadora, basándonos en que *M. strigata* es una especie que vive tanto en aguas de alta turbidez como en aguas limpias. Hay obviamente otras estructuras en el estómago que contribuyen a que esta función sea llevada a cabo con mucha eficiencia; este es el caso de un tiflosol mayor muy largo y de una posible área de selección en el capuchón dorsal; la presencia muy desarrollada del área posterior de selección con su respectivo ciego, y otros caracteres no menos importantes como son: el área del escudo gástrico con pequeños dientes quitinosos, no descrita en otros mitílidos y que representa una estructura poderosa en la desintegración de particulas; el intestino muy largo con un tiflosol bien desarrollado; y, por último, la salida separada del saco del estilo y del intestino, de manera similar a lo observado en *M. charruana* y *Musculista senhausia* descritos por Narchi & Galvão-Bueno (1983) y Morton (1974), respectivamente.

RESUMEN

Utilizando principalmente muestras fijadas de ejemplares colectados en las lagunas costeras mexicanas de Nuxco, Chautengo y Cuyutlán se describe la morfología de la concha y de las partes blandas, especialmente del estómago, en *Mytella strigata.* Se le compara con las descripciones publicadas de *M. charruana* y especies de mitílidos de los gé-

neros *Modiolus, Mytilus, Lithophaga, Perna, Arcuatula* y *Botula.* Al igual que *M. charruana* posee sifones del tipo A (Yonge, 1948), ctenidios del tipo B(1) (Atkins, 1937) y el estómago es del tipo III (Purchon, 1957) con mecanismos de selección de tipo B (Reid, 1965). Se discute la posible relación entre adaptaciones morfológicas y sus hábitos de vida.

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LABORATORY EXPERIMENTS ON THE INFLUENCE OF FOOD AVAILABILITY, TEMPERATURE AND PHOTOPERIOD ON GONAD DEVELOPMENT IN THE FRESHWATER MUSSEL DREISSENA *POLYMORPHA*

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ABSTRACT

(1) Two groups of the mussel *Dreissena polymorpha* were collected in March (E1) and September (E2). Each group consisted of individuals at different stages of the annual gonadal cycle (e.g. females: in E1 had high numbers of oocytes still increasing in size, females in E2 had empty but recrudescent gonads). (2) Each group was kept for three months under nine different combinations of temperature (5°, 12°, 19°C), food availability (high and low), and photoperiod (LD 16:8 and 8:16). At each temperature (except at 5°C with E1), a high availability of food resulted in significantly larger gonads. (3) When food availability was high, the maximum gonad volume always occurred at 12°C. A low availability of food caused a progressive decrease in gonad volume at increasing temperatures. An influence of the photoperiod (tested at low food availability at all three temperatures) was not established. (4) The increase in oocyte size to maturity in the E1-series correlated positively with increasing temperatures. Simultaneously, the number of oocytes decreased significantly, and it appears that oocytes were reabsorbed in order to support maturation of the remaining oocytes. (5) An ample supply of food influenced oocyte development positively. No influence of photoperiod was observed. (6) The results are discussed with respect to the spread of *D. polymorpha.*

Key words: *Dreissena polymorpha,* reproduction, laboratory experiments, gonads, oocyte size frequency distribution, temperature, food availability, photoperiod.

INTRODUCTION

The recent introduction of the zebra mussel *Dreissena polymorpha* (Pallas) into North American rivers and lakes (e.g. Hebert et al., 1989; Roberts, 1990) has evoked the increased interest of ecologists and water utilities as well as the general public in this "pest" species. As in Europe some 30 years ago, zebra mussel research in North America has until now focused mainly on the ability to disrupt in water supplies and influence the aquatic food web (cf. Nalepa & Schloesser, 1993). In the 1970s, the focus of research in Europe shifted to the physiological ecology of this interesting freshwater species, particularly in the context of their potential use in biomonitoring studies (Neumann & Jenner, 1992).

Research into a species' reproduction is essential for an understanding of its ecology and thus, of its ability to spread (Sastry, 1979). Among the freshwater bivalves, *D. polymorpha* is the only species to reproduce via a pelagic larva. This type of reproduction, which is common in marine species, is characterized by high fecundity, and is probably one reason as to why the zebra mussel has been able to spread rapidly in lakes and rivers presenting favourable conditions (Sprung, 1989). In Europe, the zebra mussel has colonized waters with various trophic and temperature conditions (e.g. Stánczykowska, 1977). As reviewed by Sastry (1979) for marine bivalves, both temperature and food availability can influence the course of the annual gametogenetic cycle, the rate of oocyte development, the number of differentiating oocytes (and hence the gonad size), and the onset of spawning. This is exemplified by the bay scallop *Argopecten [= Aequipecten] irradians,* where a minimum temperature of 20°C and an abundance of food are essential for the successful maturation of oocytes (Sastry, 1968).

The influence of environmental conditions on the reproduction of *D. polymorpha* is poorly understood. Onset of the spawning season in spring was correlated with a temperature threshold of about 12°C at three different locations in two central European lakes (Borcherding, 1991). In the upper hypolim-

TABLE 1. Mean daily level of energy for each mussel (Joule/day, see text) under the various conditions employed during the experiments. E1 = experiment with mussels before the spawning season, E2 = experiment with mussels after the spawning season, HFL = high food level, LFL = low food level.

	food level	photo-period	temperature (°C) 5	12	19
	HFL	LD 16:8	3.4	13.3	19.3
E1	LFL	LD 16:8	0.2	0.5	0.9
	LFL	LD 8:16	0.2	0.5	0.9
	HFL	LD 8:16	6.2	13.6	20.0
E2	LFL	LD 8:16	0.2	0.6	0.9
	LFL	LD 16:8	0.2	0.6	0.9

nion of lakes, where there is a phase shift in annual temperature cycles, the onset of spawning was delayed until late summer. However, food availability may also affect gonad development, since reductions in oocyte numbers and delays in maturation of the first oocyte cohort were observed where only a relatively low level of food was available (Borcherding, 1991). The aim of this study was to investigate the influence of food availability, temperature and photoperiod on quantitative aspects of gonad growth and oocyte development in *D. polymorpha.* This information forms a suitable basis for furthering our understanding of how the zebra mussel might become more widespread not only in Europe, but also in North America.

MATERIAL AND METHODS

Sampling and Storage

In order to conduct experiments with animals at different stages of gonad development, mussels were collected from the Fühlinger See at a depth of 2–3 m (FS-2m) before (30 March 1987-experiment E1) and after (21 Sept. 1987-experiment E2) the spawning season (Borcherding, 1991). In the laboratory, the mussels were cleaned, divided into nine groups of about 70–90 individuals, and stored in well-aereated aquaria (30 l) filled with deionized water containing 1% sea water and calcium chloride (final concentration = 160 mg Ca/l). The water was changed at least every ten days during the course of each experiment (approx. three months). At the same time, microbial growth was removed from the mussels and from the aquarium walls.

Experiments

The range of conditions used in the various experimental treatments are outlined in Table 1. *Chlamydomonas rheinhardtii* served as food and was added once per day. The amount of food available per day for each mussel was calculated as follows: the number of cells added each day and the number of mussels per day in a given aquarium was summed for the total experimental phase. As investigated in two control experiments without mussels, 25% of the algae were assumed to be unavailable to the mussels due to their sedimentation. The sum of the cells was divided by the sum of the mussels giving the mean number of cells available to each mussel per day. This value was multiplied by the energy content of *C. rheinhardtii* (weight $4.6 \pm 0.12 \times 10^{-11}$ g/cell, with about 50% organic C and an energy content of 45 J/mgC [Finlay & Uhlig, 1981], this equals 1.04×10^{-6} J/cell,), yielding the mean availability of energy to each mussel per day (Table 1). The quantity of algae at the high food level (HFL) should have covered the metabolic demand together with an additional amount of energy for growth. The metabolic demand was calculated after respiration data (for methods, see Sprung & Borcherding, 1991) obtained from mussels of the Heider Bergsee that ranged from about 50 µl O_2/day in winter to about 500 µl O_2/day in early summer (for a mussel of 20 mm shell length; Sprung, unpubl. data). These values equals about 1 to 10 J/day, following the conversion of Wieser (1986) with 20.3 J per ml oxygen. The HFL was equivalent to the mean availability of food in the Heider Bergsee, a lake with suboptimal feeding conditions (Sprung, 1989; Borcherding, 1991). The low food level (LFL) was about 5% of the HFL and should thus

have represented starvation conditions. Increases in the availability of energy with increasing temperatures were based on the respiration data named above, from which the increases in the metabolic requirements of *Dreissena* were calculated for the experimental temperatures. Experiments were performed under both long and short day conditions (LD 16:8 and LD 8:16; Table 1).

Histological Procedures and Measurements

Histological techniques had to be used to estimate gonad size on account of the close association between the gonads and other tissues of the visceral sack (Borcherding, 1991). For each experimental group, the soft portions of 16 mussels were divided into the visceral sack (gonads, digestive gland, stomach, parts of the digestive tract and adductor muscle, byssus gland) and the remaining body tissues (gills, mantle etc.). Each visceral sack was fixed in Bouin-Allen's fluid, dehydrated and embedded in paraffin. The visceral sack was sectioned transversally with a Leitz microtome. Up to 20 sections (10 µm) were taken continuously along the visceral sack. These were stained with Mayer's haemalaun and eosin and mounted in Canada balsam (Adam & Czihak, 1964).

The areas of the gonads and the entire visceral sack from each section were measured with an image analysis system (SIS GmbH, Münster). The mean tissue areas for each mussel were multiplied by the length of the visceral sack (distance from the first to last section) in order to calculate the volume of the gonads (GV), the volume of the visceral sack (VV) and the gonad index (GI = proportional volume of the gonads in the visceral sack).

The image analysis system was also used to evaluate sections of gonad tissue from each female in experiment E1. The diameters of 150 to 200 oocytes with clearly visible nuclei (to ensure that each section passed through the centre of the oocyte) were measured. The proportional volume of gamete in gonad tissue was estimated from the mean of 10 single gray-scale analyses of each female. Knowing the gonad volume (see above), these data were then used to calculate the number of oocytes per female. For the mature oocytes (diameter range 40–65 µm) identified within the gonads, a mean oocyte diameter was computed for all sizes above 40 µm. Otherwise, the mean diameter of all oocytes was calculated. Details regarding these measurements and calculations are given in Borcherding (1990).

Data Evaluation and Statistical Procedures

Gonad size and the size of other morphometric and physiological parameters of iteroparous molluscs are usually closely related to body size (reviewed by Bayne & Newell, 1983). In order to obtain estimates for a mussel with a standard size of 20 mm shell length (SL), regressions of the original data for GV, VV, GI and the corresponding SL were fitted to the allometric equation $y = aSL^b$; y is the predicted value for GV, VV or GI, as appropriate, which was calculated for a standard animal using the parameters a and b. The regressions, with 95% confidence intervals, were calculated according to Sachs (1984).

Since a regression of oocyte numbers against SL was not possible, the "change in oocyte numbers" (CON) during each experiment was estimated as follows. A theoretical number of oocytes was calculated using the SL of each female analysed at the end of the experiments and the regression equation for oocyte numbers in the initial population (theoretical number of oocytes = $30.72*SL^{3.14}$; Borcherding, 1990). The differences between the number of oocytes in the females at the end of the experiment and the theoretical number of oocytes yields the CON.

A lack of any overlap between 95% confidence intervals usually allowed a simple statistical investigation of the differences between two means (Sachs, 1984). Otherwise, the means were compared pairwise with the non-parametric Mann-Whitney U-test. The influence of various biotic and abiotic factors on the parameters measured was evaluated by analysis of variance (four-way-ANOVA or three-way-ANOVA). As in all cases the investigated variables depended on a covariate, the equality of variance was tested with the F_{max}-test of Hartley for the remaining variance of the certain regressions (Underwood, 1981; Sachs, 1984). The results of this test never showed any significant differences between the variances of the certain groups ($p > 0.05$). Every ANOVA was followed by a second one, in which the non-significant factors were excluded if the probability of the F-ratio increased to more than 0.25 (Underwood, 1981: 587–588). The final ANOVA of each experiment was followed by multiple classifica-

tion analysis (MCA), which evaluated the direction and strength of these influences (Schuchard-Ficher et al., 1982). The statistical procedures were calculated on a personal computer using SPSS/PC+ (Uehlinger, 1988).

RESULTS

The mean rate at which shell length increased in each experimental group was 1.8 µm/d (range 0–9.4 µm/d) over three months. This rate was low compared to that of populations in natural environments (e.g. up to 75 µm/d in the Ijsselmeer, Bji de Vaate; 1991; up to 60 µm/d in the River Rhine, Jantz & Neumann, 1992). Further, there was no tendency towards lower rates of shell growth in larger mussels (e.g. Bji de Vaate, 1991; Jantz & Neumann, 1992), and there was no relationship between shell growth and temperature or food availability. Mortality was generally low (0–2%) in the course of both experiments. An exception was during the first four weeks of one experiment (E1:19°C, HFL, LD 16:8, mortality = 38%), the reason for which was not apparent.

Experiments with Mussels Before the Spawning Season (E1)

At the time of their collection (end of March), mussels had well-developed gonads in the final stage of gametogenesis. Water temperature at the sampling site was about 5°C when the experiments at 5°, 12° and 19°C were run. After 90 days, gonad volume (GV) and the gonad index (GI) of mussels kept at 19°C were significantly lower than those of mussels at lower temperatures (U-test, $p < 0.05$). Under low food conditions (LFL), both GV and the visceral sack volume (VV) decreased as temperature was increased (Fig. 1). Under conditions of high food availability (HFL), GV and GI were significantly higher at 12°C than at the higher and lower temperatures (Fig. 1). If the results obtained under different experimental conditions at each temperature are compared, the positive influence of HFL on the gonads was significant only for groups at 12°C (U-test, $p < 0.05$). The results from short-day conditions (LD 8:16, tested only at LFL, data not shown) never differed significantly from those under long-day conditions.

In the experiments with mussels before

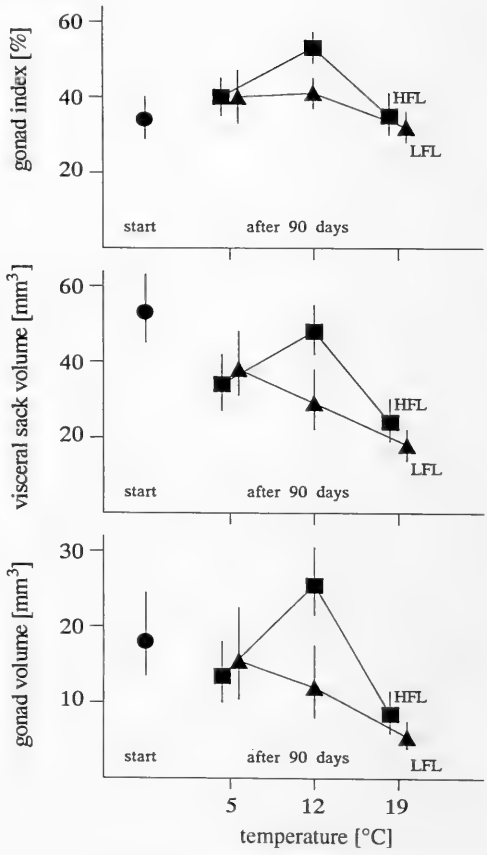

FIG. 1. *Dreissena polymorpha* (20 mm shell length): Gonad volume, visceral sack volume and gonad index of a standardized specimen at the start (30 March 1987) and end (30 June 1987) of the experiment in relation to temperature and food level under the long photoperiod (LD 16:8). All values were calculated using regression analysis, resulting in the asymmetrical 95% confidence intervals indicated by the vertical lines.

(E1) as well as after the spawning season (E2) the four-way-ANOVA confirmed that there was no significant influence of the photoperiod (probabilitity of the F-ratios 0.352 to 0.614). After Underwood (1981), those factors can be excluded from the ANOVA, in which the significance of the F-values is $p > 0.25$ (in E1 this was also the case for the sex with $p = 0.714$ [GV] and $p = 0.646$ [GI], respectively). Thus, the results of E1 were finally investigated with a two-way-ANOVA (Table 2). For GV and GI the total variance of all measurements ($n = 96$) was significantly influenced by all factors considered in the

TABLE 2. *Dreissena polymorpha:* summary statistics for the two-way-ANOVA and MCA, for all data (n = 96 for each group of values) in E1, describing the influence of the various factors on the dependent parameters GV and GI.

	gonad volume (GV)		gonad index (GI)	
	significance of F	percentage of total variation accounted for	significance of F	percentage of total variation accounted for
covariate (SL)	$p < 0.001$	36.4	$p < 0.001$	11.2
temperature	$p < 0.001$	19.4	$p < 0.001$	27.0
food level	$p = 0.397$	—	$p = 0.038$	2.9
2-way interactions	$p = 0.172$	—	$p = 0.052$	—
multiple r^2		0.561		0.412

two-way-ANOVA ($p < 0.001$). Because there were no significant two-way interactions for both variables ($p > 0.05$) and because they operate independently on the variables of interest (Underwood, 1981), the influence of the main factors can be discussed without any restrictions. Apart from the expected significant influence of shell length as a covariate, temperature was the main influence on variability in both sets of data (Tab. 2). As shown above, the MCA confirmed that a temperature of 12°C had the greatest influence on the GV, and on the GI in particular (Fig. 2). HFL had only a low, but nevertheless significantly positive, effect on the GI (ANOVA; Table 2).

Factors considered in the ANOVA accounted for 56.1% of the total variance for all the GV values, whereby 36.4% of the variance was contributed by SL and 19.4% by temperature. About 44% of the total variance was due to other factors not considered in this analysis. With GI, this proportion of unaccounted variation was almost 59%, temperature was the main influence (27%), followed by SL (11.2%). Only a small proportion of the variance was accounted for by the availability of food (2.9%; Table 2).

Analysis of the oocytes was used to provide information on factors inducing the different stages of maturity, and whether these factors might correspond to those controlling gonad size. Even though the oocytes of each mussel can vary to a certain extent under any given set of conditions, a description of the developmental tendencies may assist the recognition of environmental influences. The basis of the following classification into three types was a comparison of the oocytes at the beginning and end of each experiment.

Type 1 (minor changes): After three months, the oocyte size frequency distributions showed no clear deviation from the unimodal distribution in the initial population. The mean diameter of all the oocytes altered only slightly. Type 1 was found only in specimens from the experimental groups at 5°C and 12°C (Fig. 3), with no significant differences between these temperatures. HFL had a slightly positive influence on the mean oocyte diameter (OD) and change in oocyte numbers (CON) during the course of the experiments.

Type 2 (occurrence of mature oocytes): Mature oocytes were clearly visible in the histological slides. There was a distinct peak in numbers of large oocytes prior to spawning (Fig. 3). Mature oocytes were not identified in mussels at 5°C. Oocyte maturity remained almost constant at this temperature. At 12°C about 36% of females had a ripe oocyte cohort, and at 19°C all the females, other than a few individuals of type 3 (see below) belonged to type 2. In the groups containing ripe oocytes at the end of the experiments at 12°C and 19°C, LFL resulted in a somewhat smaller OD and a further reduction in oocyte numbers (CON) in comparison to HFL. A further reduction in the number of oocytes, up to their total elimination, was evident from the latter parameter for mussels at 19°C, compared with those at 12°C (Fig. 3).

Type 3 (reduction in oocyte size): There were no oocytes in the larger size classes, and the mean oocyte diameter decreased significantly. Up to three mussels in each group (except 12°C, HFL, LD 16:8) had significantly reduced numbers of oocyte. There was no relationship to the level of food availability or photoperiod (not shown in Fig. 3).

Since a significant negative correlation was found between OD and CON (Spearman

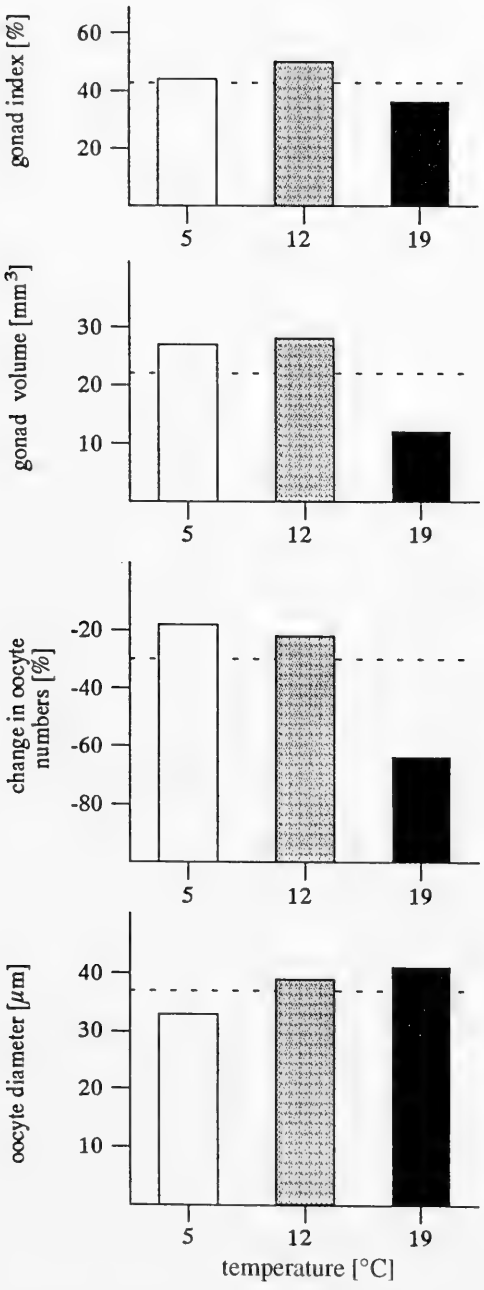

FIG. 2. *Dreissena polymorpha:* Mean values for gonad index, gonad volume, change in oocyte numbers, and oocyte diameter at three temperatures along with the overall means in E1 (dotted lines). All values were adjusted for the independent factors and covariates given in Tables 2 and 3.

Rank Correlation, p = 0.0002), CON was used as the covariate for OD and vice versa. As for the GV and GI, the photoperiod had no significant influence, and thus, this factor was excluded from the finally investigated ANOVA. There were no significant two-way interactions of the main effects (Table 3). Overall, the two-way-ANOVA demonstrated the significant effects of all the factors as well as the covariates on both these variables ($p < 0.001$). In addition to the distinct influence of the covariates (25.2% and 21.8%, respectively), both temperature and availability of food affected oocyte development significantly. Altogether, these factors accounted for about 47% of the variance in all the measurements (n = 60, for details see Table 3). The MCA indicated that an increase in temperature resulted in an increase in oocyte size, accompanied by a decrease in oocyte numbers (Fig. 2). Furthermore, the positive influence of HFL was clearly valid for OD, and to a lesser extent to CON.

Experiments with Mussels After the Spawning Season (E2)

Mussels used in this experiment were collected about one month after the spawning season (during the mussels' resting stage, cf. Borcherding, 1991) from FS-2m when the water temperature was 18.6°C. During the 90 days of this experiment, the mussels were either maintained at this temperature or at reduced temperatures of 12°C and 5°C (Table 1).

For GV and GI the total variance of all measurements (n = 96) was significantly influenced by all the factors considered in the three-way-ANOVA ($p < 0.001$, photoperiod excluded after the four-way-ANOVA because $p = 0.461$ [GV] and $p = 0.366$ [GI], respectively). As there were no significant two-way interactions or even three-way interactions for both variables ($p > 0.05$), the main factors should operate independently on the variables (Underwood, 1981). At all temperatures, gonads in the mussels at HFL were significantly larger than those at LFL (Fig. 4, below). This result was confirmed for GV by the three-way-ANOVA ($p < 0.001$, Table 4). Apart from the significant influence of shell length as a covariate on both variables ($p < 0.001$), temperature showed nearly the same effect on gonad growth as in the experiment with mussels prior to spawning (E1). The maximum value at 12°C occurred for GI un-

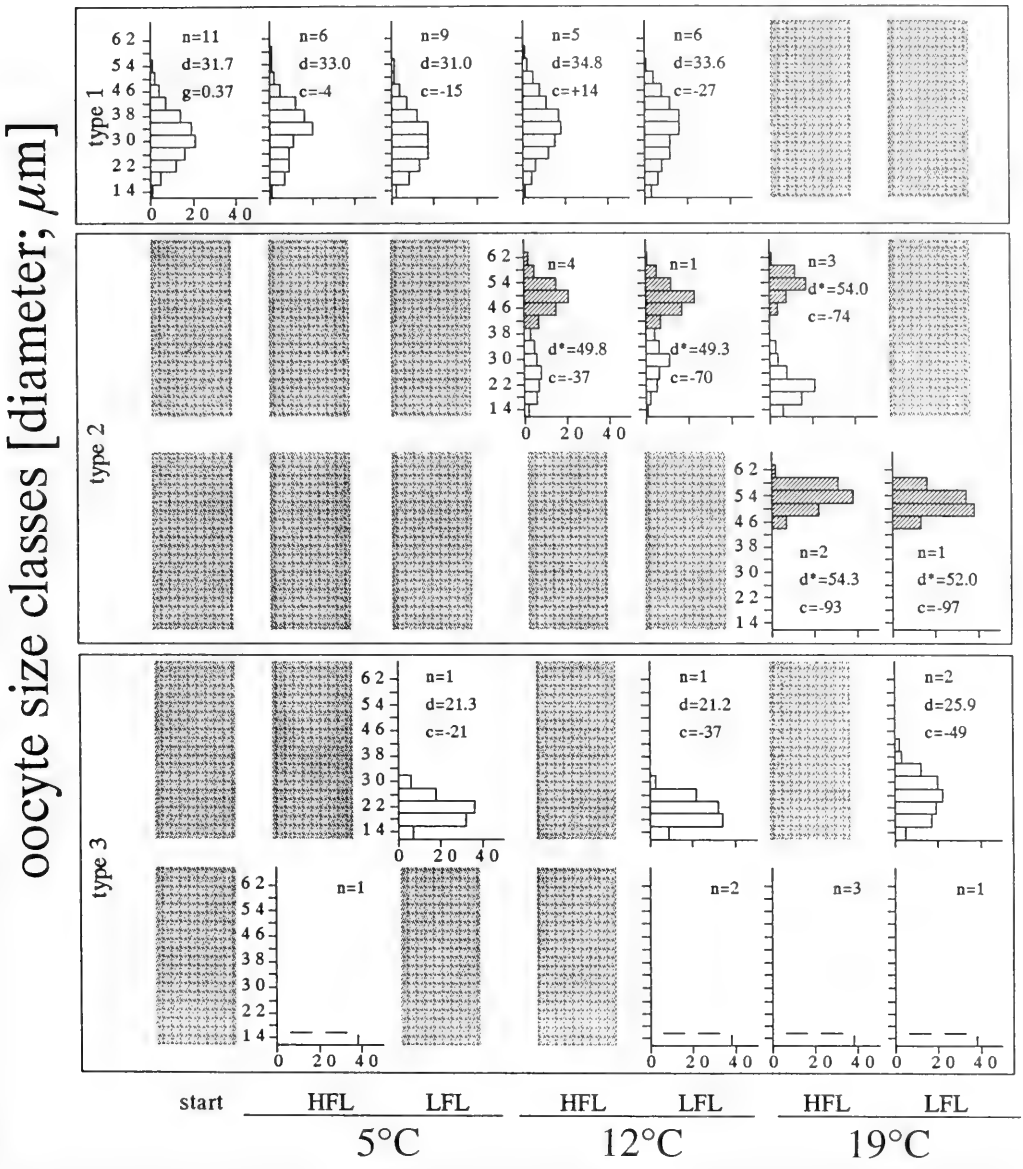

FIG. 3. *Dreissena polymorpha* (20 mm shell length): Relative frequency of oocytes in the oocyte size classes (y-axes show 4-µm classes) of standardized specimens at the start of the experiment (30.3.87) and the different conditions in E1 at the end of the experiment (30.6.87). Types 1, 2, 3: are explained in the text. n = number of females studied, d = mean oocyte diameter for all oocytes, d* = mean diameter of oocytes in the mature fraction only (stripped bars), g = total number of oocytes per mussel ($\times 10^6$) in the initial population; c = CON, the change in the total number of oocytes relative to the initial population (%). If an exact analysis of the oocytes was not possible, the histograms were estimated and are presented with hatched lines.

der all conditions (three-way-ANOVA: $p < 0.001$) and GV under HFL conditions (Fig. 4; three-way-ANOVA: $p < 0.001$, Table 4). The decrease in GV in mussels under LFL conditions at increasing temperatures also corresponds with the results of experiment E1. Overall, there were distinct similarities between the trends found in experiments E1

TABLE 3. *Dreissena polymorpha:* summary statistics for the two-way-ANOVA and MCA, for all data (n = 60 for each group of values) in E1, describing the influence of the various factors on the dependent parameters OD and CON.

	oocyte diameter (OD)		change in oocyte numbers (CON)	
	significance of F	percentage of total variation accounted for	significance of F	percentage of total variation accounted for
covariate	$p < 0.001$	25.2	$p < 0.001$	21.8
temperature	$p = 0.016$	11.6	$p = 0.001$	20.3
food level	$p = 0.003$	9.6	$p = 0.040$	4.8
2-way interactions	$p = 0.374$	—	$p = 0.420$	—
multiple r^2		0.464		0.469

and E2. Only food availability had a stronger effect in E2, especially at 5°C.

DISCUSSION

The extent to which a species can spread, as well as its success in a given environment, is related mainly to those factors which can limit reproduction (Sastry, 1979). Often the causal relationship between these limiting factors and such physiological processes as reproduction can only be evaluated in controlled laboratory experiments since the multitude of environmental factors in the field may conceal the true relationships. Bayne (1976) named three main aspects of reproduction limited by environmental factors: gametogenesis, larval development, and metamorphosis into a young adult. The first stage of reproduction, gametogenesis, creates the source of material for the subsequent steps, and should be described not only quantitatively (e.g. gonad size) but also qualitatively (e.g. stage of maturity).

Gonads

As expected for experimental groups with limited food availability, the gonad size of *D. polymorpha* under these experimental conditions was always significantly smaller than in the field population at the start of the experiment. In order to compensate for the increased metabolic requirements with increasing temperatures, the supply of energy at 19°C was about 4.5 times that at 5°C (corresponding to a three-fold increase in the energy requirement for a temperature increase of 10°C). Despite this, gonad volume still decreased with increasing temperatures at the low food availability (Figs. 1, 4). Thus it is possible that the increased food supply was not sufficient to compensate for the increased metabolic demands at higher temperatures. The decrease in gonad volume in mussels at 19°C, together with the high level of food availability indicated a similar trend.

Despite these reservations in interpreting the data, the following trends were observed. The largest gonad volumes were measured in both experiments after three months at 12°C (although only three temperatures were tested) and at high food availability (Figs. 1, 4). This shows that the results were basically independent of the initial stage of gonad development. The increase in the gonad index during the course of the experiment in nearly all the groups of mussels kept at 5°C and 12°C indicated intensified gonadal growth compared with other tissues, at lower temperatures. In addition, and despite the reservations outlined above about the interpretation of the data at 19°C, the distinct negative influence of higher temperature on gonad volume was revealed.

The statistical analysis showed that gonad volume and the change in oocyte numbers were influenced in a similar manner (e.g. for temperature, see Fig. 2). This means that gonad volume was mainly a function of the number of oocytes. Thus it should be possible to compare the results of the present study with measurements of eggs spawned by *Mytilus edulis* after storage under various conditions, as reported by Bayne et al. (1978). These authors demonstrated a higher fecundity in mussels at 11°C than at 18°C, and a reduction in the number of eggs spawned when no food was available. Together with estimations of the "scope for growth," Bayne et al. (1978) concluded that

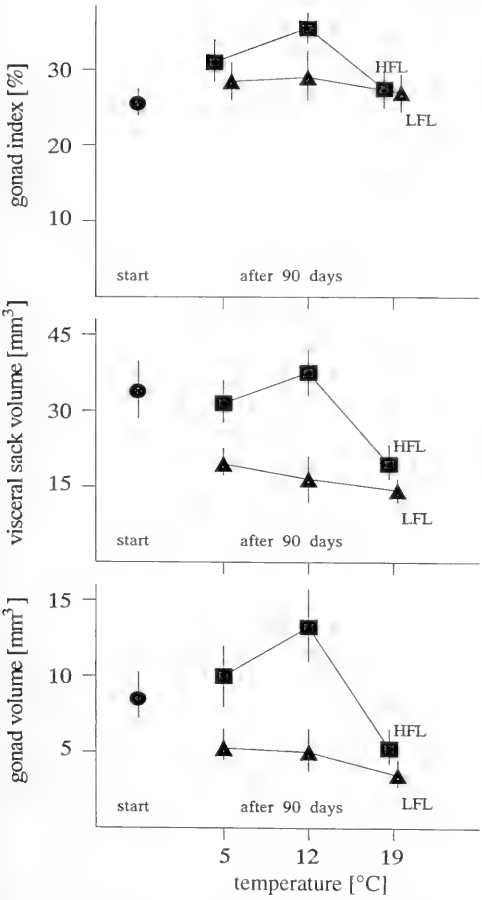

FIG. 4. *Dreissena polymorpha* (20 mm shell length): gonad volume, visceral sack volume, and gonad index of a standardized specimen at the start (21 Sept. 87) and end (23 Dec. 87) of the experiment, in relation to temperature and food level under the short photoperiod (LD 8:16). All values were calculated using regression analysis, resulting in the asymmetrical 95% confidence intervals indicated by the vertical lines.

fecundity in *M. edulis* depends mainly on the energy available for gamete production. This conclusion seems to be valid for *D. polymorpha* as well because the results indicate that higher fecundity (i.e. gonad size or number of oocytes) was related to a sufficient supply of energy.

Sastry (1968), working on *A. irradians,* reported slightly higher gonad indices (the contribution of gonad to the body weight) at 15°C and 20°C in fed mussels, compared to starved mussels. However, these differences were not significant. In the present study, gonad indices based on volume or weight were not sufficient to reveal all the trends. For example, if E2 values for the gonad index only were taken into account, then the significant differences between gonad volume in the mussels at HFL and LFL at 5°C and 19°C would not have been recognized (Fig. 4). The conclusive evidence for the significant influence of food availability on gonad development could only be drawn from the absolute values (Table 4). On the other hand, the similarity in gonad indices, along with different gonad volumes (e.g. E2: 19°C conditions, Fig. 4), implies that the gonads are supported at the cost of other tissues under conditions of environmental stress (i.e. low food availability).

In contrast to temperature and food availability, photoperiod never had a significant influence on the gonad development in *D. polymorpha* (Tables 2–4). The results of E2 (Fig. 4) were similar to those of Gimazane (1971, cited by Sastry, 1979), who found that photoperiod had no significant effect on gametogenesis in *Cerastoderma [= Cardium] edule* with gonads at the resting stage. Photoperiod also had no influence on the gonads and oocytes in *D. polymorpha* at the end of gametogenesis (E1: Tables 2, 3). However, Bohlken & Joosse (1982) reported that an LD 16:8 induced a relatively early maturation of the female reproductive system and a high rate of egg production in the gastropod *Lymnaea stagnalis.* The possibility of photoperiod inducing similar effects in the zebra mussel, for instance under conditions of high food availability or longer experimental periods, can only be clarified with appropriate experiments.

Oocytes

Information on the gonad size only is not sufficient for assessing the different stages of maturity. A better evaluation is provided by the mean oocyte diameter (of either all the oocytes or just the ripe oocytes), which can be used to approximate the stage of maturity (Sastry, 1979). Food availability influenced oocyte size in the same manner as gonad volume, but the influence of temperature was totally different on both the above-mentioned variables in *D. polymorpha* from E1. Oocyte diameter, which is related to the stage of maturity, increased with temperatures (Fig. 3). This was the opposite effect to that witnessed for gonad size and the change in

TABLE 4. *Dreissena polymorpha:* summary statistics for the three-way-ANOVA and MCA, for all data (n = 96 for each group of values) in E2, describing the influence of the various factors on the dependent parameters GV and GI.

	gonad volume (GV)		gonad index (GI)	
	significance of F	percentage of total variation accounted for	significance of F	percentage of total variation accounted for
covariate (SL)	$p < 0.001$	48.3	$p < 0.001$	23.9
temperature	$p < 0.001$	9.6	$p = 0.001$	13.0
food level	$p < 0.001$	9.0	$p = 0.049$	2.9
sex	$p = 0.051$	—	$p = 0.185$	—
2-way interactions (t-f)	$p = 0.051$	—	$p = 0.428$	—
2-way interactions (t-s)	$p = 0.893$	—	$p = 0.831$	—
2-way interactions (f-s)	$p = 0.518$	—	$p = 0.669$	—
3-way interactions	$p = 0.875$	—	$p = 0.867$	—
multiple r^2		0.672		0.399

oocyte numbers (Fig. 2). This indicates that the maturation of oocytes, even if it was only a low portion of the total number, was restricted to higher temperatures (a minimum of 12°C for the temperatures tested).

Similar conclusions have been drawn for *Crassostrea virginica* (Loosanoff & Davis, 1952) and *A. irradians* (Sastry, 1966). Sastry (1968) reported that bay scallops from North Carolina developed oogonia when exposed to a sub threshold temperature of 15°C, but oocyte growth did not occur even though they were supplied with ample food. After transferring these scallops to higher temperatures (20°C and 25°C), oocyte growth began immediately when sufficient food was available (Sastry, 1968). However in bay scallops from Massachusetts, the cytoplasmatic growth phase of oocytes was initiated at 15°C, at 5°C only oogonia developed (Sastry & Blake, 1971). Sastry (1970) suggested that such variations between populations may be an adaptive response to geographic differences in temperature and food production.

In contrast to *A. irradians,* the zebra mussel was able to develop only a fraction of its oocytes to maturity at temperatures of 12°C (Fig. 3), even when the availability of food was so low that gonad size was reduced to less than 30% of its initial value (Fig. 1). This might occur at the expense of body reserves (e.g. in *M. edulis,* Gabbott & Bayne, 1973; Gabbott, 1975). On the other hand, the decrease in oocyte numbers and the maturation of parallel oocyte cohorts (Fig. 3) suggested that some of the oocytes were reabsorbed in order to support a remaining, smaller portion of the oocytes. The possibility of oocyte resorption in *D. polymorpha* was discounted by Walz (1978), although gonad size and oocyte numbers were not evaluated, casting a doubt on the conclusions of Walz' study. The resorption of oocytes in response to environmental stress is a common adaptation in many bivalves (e.g. *C. virginica,* Loosanoff & Davis, 1951; *A. irradians,* Sastry, 1966; *M. edulis,* Bayne et al., 1978, 1982). It was also described recently in *D. polymorpha* during periods of starvation (Sprung & Borcherding, 1991), with electron microscopy providing direct evidence for resorption processes during the same experiments (Bielefeld, 1991).

Oocytes in the mussels studied in E2 were poorly developed at the end of the experiment, which made an extensive analysis of the oocytes difficult. Using microscopy, it was possible to gain an impression of the stage of oocyte development. This confirmed that nearly all the trends outlined above for the gonad volume (maximum values at 12°C, minimum values at 19°C, a negative influence of LFL) were also valid for the stage of oocyte maturity. This is in clear contrast to the situation in E1 (Fig. 2). In autumn, at the onset of the gametogenetic cycle in *D. polymorpha,* the increase in gonad volume could be attributed mainly to the proliferation of new germ cells, and only to a small extent to the growth of oocytes (Borcherding, 1991). An identical situation occurred with the mussels in E2. Gonad size increased but, in contrast to E1, the oocytes did not mature at 12°C and 19°C. Two factors may have been responsible: (1) an endogenous component and/or (2) the necessity for low temperatures during a certain phase of the annual repro-

ductive cycle, perhaps to initiate or synchronize certain gametogenetic processes in *D. polymorpha*.

To summarize, it appears that fecundity (i.e. number of oocytes, gonad volume) was influenced mainly by food availability under the different experimental conditions, both in spring and in autumn. However, maturation of the oocytes (i.e. their size) was affected positively by increased temperatures only in spring prior to spawning, while in autumn there was no effect on the stage of maturity for mussels at the onset of gametogenesis.

Conclusions

(1) Temperature: Borcherding (1991) pointed out that environmental temperature across the year may limit the further spread of *D. polymorpha* in three ways. First, if temperatures remain above 12°C throughout the year, oocyte maturation and spawning may become desynchronized within a population (possibly the stimulus for initiating maturation is lacking), thus an important prerequisite for fertilization with this type of reproduction would be lost. Second, temperatures fail to rise above the apparent threshold of 12°C in cold monomictic lakes, then spawning cannot occur and fertilization of the eggs would not be possible (Sprung, 1987). Third, a low amplitude in annual temperatures might be insufficient for temporal control (e.g. deep-sea species, Mackie, 1984), whereas high amplitudes might be unfavourable if rates of respiration and ingestion are not balanced during periods of rapidly changing temperatures (Bayne & Newell, 1983). The results of the experiment with mussels at the onset of gametogenesis (E2, 19°C) appear to correspond with the first suggestion. Although high temperatures can support oocyte maturation (if gonads are in another phase of the gametogenetic cycle, cf. E1), the growth of oocytes was reduced at high temperatures. Possibly a spell of low temperatures is necessary after spawning in order to initiate a new gametogenetic cycle. On the other hand, the results of E1 revealed that oocytes did not mature at low temperatures (5°C), thus higher temperatures (here a 12°C threshold) are required prior to spawning, which confirms to the second of these suggestions.

(2) Food Availability: Fecundity in *D. polymorpha* was undoubtedly reduced under conditions of low food availability (cf. Borcherding, 1992). This was confirmed by the increased reduction in oocyte numbers under reduced food availability (indicated in E1). However, low food availability alone, with no unfavourable temperature conditions (e.g. rapidly changing temperatures), was not sufficient to prevent the first stage of reproduction. The results of E1 and other starvation experiments (Sprung & Borcherding, 1991) showed that a small portion of the oocytes in *D. polymorpha* could mature under adverse conditions of food availability. Thus, low food concentrations alone may restrict, but do not prevent, the spread of *D. polymorpha* in such an environment. A restriction in the spread of this species would appear to be influenced only by the ambient temperature conditions.

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RELAXING TECHNIQUES FOR FRESHWATER MOLLUSCS: TRIALS FOR EVALUATION OF DIFFERENT METHODS

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ABSTRACT

Twelve different methods of relaxing freshwater molluscs were tested to find the most suitable for future research and conservation in scientific collections. In addition to drowning, different concentrations of the following agents were tested: phenoxyethanol, MS 222, clove oil, pentobarbital, sodium pentobarbital, diethylether, chloroform, and urethan. Also menthol, lime tree, and valerian were tried. Tests were made with species of main groups of freshwater molluscs: *Pisidium amnicum, Corbicula fluminea,* and *Unio* sp. among bivalves; *Melanopsis* sp., *Bithynia tentaculata, Valvata piscinalis, Potamopyrgus jenkinsi, Pseudamnicola* cf. *luisi,* and *Horatia sturmi* among prosobranch gastropods; and *Lymnaea peregra* and *Ancylus fluviatilis* among pulmonate gastropods. Relaxation condition of specimens after narcosis, response to fixative fluids, action time and availability of narcotic agents were considered for evaluation. There was considerable variation between species in their susceptibility to narcotic agents, suggesting that many factors may be involved in the response of freshwater molluscs to narcotization. Among tested agents, sodium pentobarbital and especially, pentobarbital, were the most suitable for relaxing freshwater molluscs. No overdosing troubles were registered in trials with pentobarbital. Results with menthol were unpredictable, although it may be used over a wide range of species.

Key words: freshwater molluscs, pulmonates, prosobranchs, bivalves, narcotization, relaxing agents, techniques, fixation.

INTRODUCTION

Anatomical studies in such areas as taxonomy, physiology, ecology and systematics of molluscs, and the increased interest in morphometrics (Meier-Brook, 1976b; Emberton, 1989), have generated a need for improved relaxing techniques for the study of molluscs. The preservation of specimens in a life-like position is also important for natural history collections.

A search of the literature yielded only a few papers dealing specifically with methods for relaxing freshwater molluscs. Runham et al. (1965) reviewed the results of previous authors while trying both narcotics and anaesthetics on different species of freshwater and terrestrial gastropods, showing that methods vary considerably in effectiveness among species. Meier-Brook (1976a) compared the effectiveness of two different agents (pentobarbital and sodium pentobarbital) on species of Basommatophora, Prosobranchia and Bivalvia. Meier-Brook (1976b) tested the effect of different levels of extension after narcosis on the anatomical measurements of a *Planorbis* species. More recently, Girdlestone et al. (1989) used different concentrations of three volatile products to reversibly anaesthetize specimens of *Lymnaea stagnalis* (Linnaeus, 1758).

The experiences of previous authors together with our own, led us to carry out tentative tests in order to obtain more information before designing the present experiment. Since the aim is to obtain properly extended animals for immediate dissection and/or fixation, selection of methods does not take into consideration whether animals might be allowed to recover, as other authors have required (Michelson, 1958; Girdlestone et al., 1989). Therefore, we employ the term narcotization in the sense of Runham et al. (1965). According to them, the best narcotic will relax the animals "in as life-like a position as possible and to such an extent that they do not contract when fixed." Therefore, we tested several narcotics in different concentrations to various species of the main groups of freshwater molluscs.

Some methods previously cited for narcotization of marine molluscs (Smaldon & Lee, 1979) or other animals (Lincoln & Sheals, 1979) are used in this paper for the first time

for freshwater molluscs. "Drowning" was also tried to test the effect of the progressive lack of oxygen on some species. From the eleven species tested, only *Lymnaea peregra* (Müller, 1774), *Bithynia tentaculata* (Linnaeus, 1758), and *Potamopyrgus jenkinsi* (Smith, 1889) were previously used in similar studies by other researchers.

The purpose of this investigation was to discover the most suitable relaxing method for each species or species group of freshwater molluscs, with special attention to the repeatibility of the procedures in future research.

Relaxation (degree of extension) of specimens after narcosis, response to fixative fluids, action time and availability of the narcotic agents, were all considered in evaluation of their effectiveness.

MATERIALS AND METHODS

Before planning a definitive protocol, we tried several products and concentrations, as well as different fixation routines and laboratory conditions. These early experiences will be called "pretests" in this paper, and the subsequent ones will be simply called "tests."

Pretests

These were carried out with menthol, adding crystals to cover the water surface of the jar, and with sodium pentobarbital (1% and 2%), urethane (2% and 4%), MS 222 (0.05%, 0.1% and 0.2%), clove oil (from 3 to 30 drops depending on the water volume), phenoxyethanol (1%), magnesium chloride (7.5%), magnesium sulphate (7.5%) and chloral hydrate (5%) among narcotic agents. The volume of the glass jars was twice that of the tested solution (7 ml, except 60 ml for *Unio* sp.) and solutions were prepared with dechlorinated tap water (for exceptions see results). Pretests were made in covered jars in a refrigerator (10–13°C). Menthol was also tested at room temperature (20–28°C). The effect of "drowning" over the specimens was proved in 15 ml of water (100 ml for *Unio* sp.). Pretests were regularly checked in order to monitor changes in the general aspect of animals. After checking the lack of response to mechanical stimulus (by use of a needle), specimens were killed by different methods: 70% ethanol at room temperature and at 12°C below zero (± 2°C), liquid nitrogen after ten seconds and hot water (60°C) after five seconds. Fixation was always carried out in 70% ethanol. Five specimens of *Potamopyrgus jenkinsi,* three of *Valvata piscinalis* (Müller, 1774), two of *Bithynia tentaculata* and *Pisidium amnicum* (Müller, 1774) and one of *Corbicula fluminea* (Müller, 1774) and *Unio* sp., depending on specimen availability, were used in each test.

Tests

The results of the pretests were used as the basis for choosing the agents and establishing the conditions for the following tests:

Several species of the main groups of freshwater molluscs were chosen in order to test if responses to narcotic agents were similar within each group. Trials were made with *Pisidium amnicum, Corbicula fluminea,* and *Unio* sp. among bivalves; *Melanopsis* sp., *Bithynia tentaculata, Valvata piscinalis, Potamopyrgus jenkinsi, Pseudoamnicola* cf. *luisi* Boeters, 1984, and *Horatia sturmi* (Rosenhauer, 1856) among prosobranch gastropods; and *Lymnaea peregra* and *Ancylus fluviatilis* (Müller, 1774) among pulmonate basommatophoran gastropods. No species of the family *Planorbidae* were tested as they had been included in studies by Michelson (1958) and Meier-Brook (1976a, b). Voucher specimens are deposited in collections of the Museo Nacional de Ciencias Naturales, Madrid, Spain.

Bithynia tentaculata, Valvata piscinalis, Potamopyrgus jenkinsi, Lymnaea peregra, Pisidium amnicum and *Corbicula fluminea* were sampled from the Miño River (Pontevedra, Spain) in January 1992. Specimens of *Unio* sp. were captured at the Gasset reservoir (Ciudad Real, Spain) in December 1991. *Pseudamnicola* cf. *luisi* and *Horatia sturmi* were sampled at Pilar del Mono fountain (Granada, Spain) in February 1992. Specimens of *Melanopsis* sp. were collected at the Molí fountain (Alicante, Spain) in January 1992, and *Ancylus fluviatilis* from the Perales River (Madrid, Spain. November, 1991). Animals were transported alive to the laboratory in plastic jars inside a portable refrigerator with ice, and artificial aeration was provided for five seconds every eight hours. At the laboratory they were kept in aquaria at 13°C with a 12-hour artificial day-night cycle and aeration until tests were carried out. The

TABLE 1. Number of days each species was kept in aquaria

	TEST	REPEATED TEST
Melanopsis sp.	10	12–24
Valvata piscinalis	6	9
Bithynia tentaculata	27	31–39
Potamopyrgus jenkinsi	13	20
Pseudamnicola cf. *luisi*	16	24
Horatia sturmi	3	—
Ancylus fluviatilis	2	3
Lymnaea peregra	6	10–18
Unio sp.	1	21
Pisidium amnicum	12	19–41
Corbicula fluminea	24	34

number of days each species was kept under this cycle varied, and is indicated in Table 1.

Pretests indicated that drowning might be effective in some species. This was proved for specimens of *Melanopsis* sp, which were submerged in water in a covered jar avoiding air bubble formation. Eleven narcotics were tested (Table 2). Five of them, sodium pentobarbital, pentobarbital, MS 222, phenoxyethanol and urethane were used in different concentrations (see Table 2). Doses of clove oil are explained in Table 2. Diethylether and chloroform were also tested with *Melanopsis* sp. because this species group proved to be very resistant to narcotization. Trials with menthol were made as in pretests, adding crystals to cover the water surface of the jar. No records were found in the literature indicating the use of valerian, lime tree, clove oil, diethyleter, chloroform, phenoxyethanol nor MS 222 in narcotization of freshwater molluscs. Tested products, and when necessary, commercial names and firms are:

Pentobarbital and sodium pentobarbital (nembutal) are manufactured by Sigma Chemical Co.

Ethyl M-Aminobenzoate is manufactured by Sandoz under the name MS 222.

Phenoxyethanol is manufactured by Merck under the name Ethyleneglycolmonophenylether.

Urethane is manufactured by Fluka Chemie AG.

Lime tree is *Tilia* sp.

Valerian is *Valeriana officinalis* (Linnaeus, 1758).

Menthol Cryst is manufactured by Merck.

Clove tree oil is from *Syzygium aromaticum* (Linnaeus, 1758).

Diethylether is manufactured by Riedel-de Haën.

Chloroform is manufactured by Probus S.A.

Water used for dilutions came from the same site as the species tested, except for *Horatia sturmi* and *Pseudamnicola* cf. *luisi,* where the water came from the Miño River and from dechlorinated tap water, respectively. Dilutions were prepared as soon as samples reached the laboratory and stored in a refrigerator at 10–13°C, being transferred to room temperature 12 hours before each experiment. The standard volume of dilutions was 8 ml, except for larger species, such as *Unio* sp. and *Corbicula fluminea,* where the volume was 150 ml and 37 ml, respectively. One specimen of *Melanopsis* sp. and *Unio* sp., two of *Bithynia tentaculata, Horatia sturmi, Pisidium amnicum* and *Lymnaea peregra,* and three of the remaining species were tested. Tests were made in covered glass jars with a total volume of 16 ml, except for *Corbicula fluminea* and *Unio* sp., in which jar volumes were of 82 and 240 ml respectively.

In order to stardardize the experiment and to avoid maceration after full relaxation, we carried out experiments at 6–10°C in all cases except in *Melanopsis* sp., where, due to difficulties to relax it, tests were also done at 15–18°C. All tests were simultaneously done for each species. Once the complete set of tests was concluded with each species, only those drugs and concentrations yielding the best results were subsequently used to repeat the experiment. Finally, the most successful method was used in a third test to narcotize all the remaining specimens of each species sample.

The experiments were regularly checked, as in pretests, in order to record any changes in the animals (with a stereomicroscope when necessary). After we checked for lack of response to mechanical stimulus, we fixed specimens in ethanol at −12°C (±2°C).

Criteria for Evaluation

The maximum extension (relaxation) achieved with each method was observed and quantified according to the following code (Tables 3–5): 4 = very good (animal fully extended and sometimes a little turgid or swollen), 3 = good (not fully extended and sometimes wrinkled), 2 = fair (only part of the foot visible outside the shell), and 1 = bad

TABLE 2. Chemical products and concentrations as weight percentage[1]

Pento-barbital	Sodium Pento-barbital	MS 222	Phenoxy-ethanol	Urethane	Lime tree	Valerian	Menthol cryst	Clove oil	Diethylether	Chloroform
0.400%	2.000%	0.20%	1.00%	2.0%	1.0%	1.0%	(*)	(**)	(***)	(***)
0.200%	1.000%	0.10%	0.50%	1.0%				15 drops		
0.100%	0.500%	0.05%		0.5%				10 drops		
0.050%	0.250%							5 drops		
0.025%	0.125%									

[1]Phenoxyethanol is as volume percentage.
(*): Enough to cover vial surface.
(**): Except for *Unio* sp (60, 40 and 20 drops) and *Corbicula fluminea* (30, 20 and 10 drops).
(***): Only tested with *Melanopsis* sp, see literature.

(animal withdrawn inside the shell). These codes refer to all specimens tested, with the exceptions quoted in the results section. In the case of 2, 3 and 4 (fair, good and very good extension), the time consumed by each method (action time) was registered and quantified as follows: 4 < 24 hours, 3 = 24–48 hours, 2 = 48–72 hours and 1 > 72 hours. The response of specimens to the fixation after one minute (fixation I) and after 24 hours (fixation II) was also codified in the case of 2, 3 and 4, as follows: 4 = very good (no retraction, without modification), 3 = good (slight retraction), 2 = fair (large retraction) and 1 = bad (animal withdrawn). All data and incidences of the experiment were registered in specially designed forms.

RESULTS

Magnesium chloride, magnesium sulfate and chloral hydrate were not successful when tested on *Pisidium amnicum, Corbicula fluminea, Unio* sp., *Bithynia tentaculata, Valvata piscinalis* and *Potamopyrgus jenkinsi,* and therefore were rejected for subsequent tests. Urethane (4%) was only successful for *Unio* sp. and was lethal for smaller species; in the remaining tests it was used at lower concentrations.

Freezing was also tried with some hydrobiid species. Frozen animals were fully expanded and did not retract when fixed in 70% ethanol, contrary to that observed by Runham et al. (1965) where calcium formalin was used. However, this technique was discarded since it resulted in serious damage to the skin of the animal and consequent loss of external morphological characters of taxonomic interest.

From all the methods employed for killing specimens, sudden immersion of relaxed specimens in hot water (60°C) before fixation, seemed most effective in avoiding animal retraction. This weakens the columelar muscle of gastropods, enabling easy extraction of the animal without breaking the shell. However, this method was not used in further tests as it is suspected of causing internal tissue disturbances. Submersion in liquid nitrogen was also rejected since in species tested (*Potamopyrgus jenkinsi, Unio* sp., and *Pisidium amnicum*) it damaged the skin of the animal. For subsequent tests, cold ethanol was used in the fixation of specimens to avoid retraction caused by ethanol at room temperature.

A complete reference to results can be found in Tables 3–5. Results on each species are discussed below for those cases when narcosis was good or very good, according to the codes specified in the previous section and in the Tables. For those species in which pretests were made, results are described following the results of the tests.

Melanopsis sp. (Table 3)

Specimens of *Melanopsis* sp. are difficult to relax. Results obtained under the same conditions as those of other species tested were very poor. Consequently, tests were repeated at different temperatures trying such additional drugs as diethylether and chloroform.

The best results were obtained using 0.25% sodium pentobarbital and 0.05% pentobarbital, narcotization being good at 48 and 72 h, respectively. Sodium pentobarbital (0.25%), yielded a very good fixation I and a fair fixation II. When this test was repeated with 15 adult specimens and four juveniles in

TABLE 3. Results with prosobranch gastropods

	Melanopsis sp.	*Valvata piscinalis*	*Bithynia tentacula*	*Potamopyrgus jenkinsi*	*Pseudamnicola* cf. *luisi*	Horatia sturmi
PENTOBARBITAL						
0.400%	3.3.4.1	2.1.2.2	3.2.4.4	4.4.4.4	3.4.4.3	4.3.4.4
0.200%	2.3.4.1	4.1.4.4	3.2.4.3	4.4.4.4	4.4.4.4	4.3.4.4
0.100%	1.-.-.-	4.1.3.3	4.1.4.3	4.3.4.4	4.3.4.4	4.3.4.4
0.050%	3.2.4.4	4.1.4.4	3.3.4.4	4.3.4.4	1.-.-.-	4.3.4.4
0.025%	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-
SODIUM PENTOBARBITAL						
2.000%	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	2.2.4.4
1.000%	2.3.4.4	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	2.2.4.4
0.500%	2.3.4.4	4.1.3.3	1.-.-.-	4.4.3.3	2.1.2.2	4.2.4.3
0.250%	3.3.4.4	4.1.4.4	4.1.4.3	4.3.4.4	4.2.4.4	4.2.4.4
0.125%	3.3.4.3	4.1.4.4	4.1.4.4	4.2.4.4	4.1.4.4	4.2.4.4
MS 222						
0.20%	1.-.-.-	3.1.4.4	2.2.4.3	1.-.-.-	2.2.4.3	1.-.-.-
0.10%	1.-.-.-	1.-.-.-	4.1.3.2	1.-.-.-	2.1.3.3	3.2.4.3
0.05%	1.-.-.-	1.-.-.-	4.1.1.1	1.-.-.-	2.1.3.3	2.1.3.3
PHENOXYETHANOL						
1.00%	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	2.2.3.3	2.3.3.3
0.50%	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	3.2.3.3	3.3.3.3
URETHANE						
2.0%	1.-.-.-	1.-.-.-	4.1.4.4	3.4.3.2	2.1.4.4	3.3.4.3
1.0%	1.-.-.-	1.-.-.-	4.1.4.2	1.-.-.-	2.1.3.3	3.2.4.3
0.5%	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-
LIME TREE						
1.0%	2.2.4.4	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	2.2.4.1
VALERIAN						
1.0%	2.2.4.4	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	3.2.4.4
MENTHOL CRIST						
(*)	3.4.2.2	2.1.2.2	2.2.4.3	1.-.-.-	3.4.4.3	4.3.4.4
CLOVE OIL						
15 drops	1.-.-.-	2.1.2.2	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-
10 drops	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-
5 drops	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-	1.-.-.-
DIETHYLETHER						
(**)	4.3.2.1					
CHLOROFORM						
(**)	1.-.-.-					
DROWNING 1						
	1.-.-.-					
DROWNING 2						
	2.1.3.3					

Results are the best obtained including repetitions. When narcotization is bad (value 1) no results of time nor fixations I and II are registered.

First column, under each species, shows narcotization values (4 = very good, 3 = good, 2 = fair and 1 = bad), second column shows action time (4 < 24 hours, 3 = 24–48 h, 2 = 48–72 h and 1 > 72h), third and fourth columns show fixation I and fixation II respectively (code values as for narcotization).

(*) Enough to cover vial surface. (**) Adding drop by drop. Drowning 1 results are with water from the same locality of the species. Drowning 2 results are with deionized water.

a solution of 100 ml, good narcotization was obtained in 72 h as well as very good fixation I and fixation II, thus equalling the results obtained with 0.05% pentobarbital.

Good narcotization and fixation can be obtained in 48 h with 0.125% sodium pentobarbital. Also good narcotization was with 0.4% pentobarbital in 48 h (poor fixation II) and with menthol in 24 h (fair fixation II).

Results with chloroform were not satisfactory, but by adding diethylether drop by drop to the water, a proper narcotization can be obtained in 48 h. However, fixation was always very unsatisfactory.

TABLE 4. Results with pulmonate gastropods

	Ancylus fluviatilis	*Lymnaea peregra*
PENTOBARBITAL		
0.400%	4.4.4.3	4.1.4.3
0.200%	4.4.4.4	4.1.4.3
0.100%	4.4.4.4	4.1.4.3
0.050%	3.4.4.2	4.1.4.3
0.025%	1.-.-.-	1.-.-.-
SODIUM PENTOBARBITAL		
2.000%	2.4.1.1	2.4.3.1
1.000%	4.4.4.4	2.4.4.1
0.500%	4.4.4.4	2.1.3.1
0.250%	3.4.3.3	4.1.4.3
0.125%	4.4.4.3	4.1.4.3
MS 222		
0.20%	3.4.2.2	2.4.3.1
0.10%	3.4.3.3	1-.-.-
0.05%	1.-.-.-	1.-.-.-
PHENOXYETHANOL		
1.00%	2.4.3.1	1.-.-.-
0.50%	2.4.4.1	3.4.3.1
URETHANE		
2.0%	4.4.4.4	3.1.4.4
1.0%	3.4.2.2	4.1.4.2
0.5%	3.4.2.2	1.-.-.-
LIME TREE		
1.0%	2.4.3.2	3.1.4.2
VALERIAN		
1.0%	3.4.2.2	2.1.3.2
METHOL CRIST		
(*)	4.4.4.4	3.4.4.1
CLOVE OIL		
15 drops	2.4.4.2	2.4.3.1
10 drops	3.4.4.2	3.4.3.1
5 drops	3.4.3.3	3.4.4.1

Conditions and codes as in Table 3.

Valvata piscinalis (Table 3)

Pentobarbital (0.05%) and 0.25% and 0.125% sodium pentobarbital at 96 h were successful. However, results of the same tests at 77 h were only fair. Pentobarbital (0.2%) worked initially very well, but in repetition it was successful in one out of three cases. Good results were also obtained with 0.5% sodium pentobarbital and 0.2% MS 222, although not all tested specimens were as well extended as with the previous methods. Pentobarbital (0.1%) gave irregular results among the specimens tested including the repetition; some specimens remained withdrawn while in others extension was very good. Time elapsed for narcotization in all these cases was between 77 h and 96 h.

A third test carried out with 0.125% sodium pentobarbital in 50 ml of solution with 48 specimens, gave very good results in 77 h (27 specimens fully extended, 13 uncompletly extended and 8 withdrawn).

Pretests were made with specimens from the same locality but collected in August 1990. Menthol and drowning tests were made in 0.5 ml of water. Results with 0.05%, 0.1% and 0.2% MS 222 were fair in 49 h, except in the last case which was good in 25 h. Results with menthol at 12°C (25 h), 2% urethane (25 h) and drowning (72 h) were also fair, whereas tests with 1% and 2% sodium pentobarbital, 4% urethane, and menthol (24–28°C) were not satisfactory.

Bithynia tentaculata (Table 3)

The best results were obtained in 54 h with 0.125% sodium pentobarbital. In the second and third repetitions, time elapsed was over 100 h. The third repetition was carried out with 50 specimens in 50 ml of solution, but the result was not as good as in the first trials because the experiment could not be completed. Fixation I and fixation II were very good in all cases.

At 72 h and 80 h, 0.1% pentobarbital and 0.25% sodium pentobarbital, respectively, yielded very good narcotization and fixation I, although animals withdrew slightly after 24 h (fixation II). The same results were obtained in both repetitions but one specimen remained closed in 0.1% pentobarbital.

Results were optimal at 80 h with 1% and 2% urethane, although fixation II was not good with the former dilution. A subsequent repetition of tests did not yield good results.

Very good narcotization and fair fixation II was obtained in 78 h and in 100 h (in the repetition) with 0.1% MS 222. With 0.05% MS 222 (no repetition was made), narcotization was very good at 80 h, but fixations I and II were very poor.

With 0.4% and 0.2% pentobarbital for 56 h results of narcotization were good for one specimen and unsatisfactory for the other, but no test was repeated. Likewise without repetition, 0.05% pentobarbital yielded good narcotization in only one specimen in 32 h. Fixations I and II were very good.

Pretests were also made with specimens from the same location but collected in June 1990. Results using 0.05% and 0.1% MS 222 were very unsatisfactory as in the case of 4% urethane. Good results were obtained using

TABLE 5. Results with bivalves.

	Unio sp.	*Pisidium amnicum*	*Corbicula fluminea*
PENTOBARBITAL			
0.400%	2.4.4.4	2.1.3.1	3.1.3.3
0.200%	3.4.4.3	2.1.4.2	4.1.4.2
0.100%	4.3.4.3	3.1.4.3	4.1.4.2
0.050%	4.3.4.3	3.1.4.3	4.1.4.3
0.025%	3.2.4.3	3.1.4.3	1.-.-.-
SODIUM PENTOBARBITAL			
2.000%	2.4.4.4	1.-.-.-	1.-.-.-
1.000%	3.4.4.4	3.2.4.4	1.-.-.-
0.500%	2.4.4.4	4.3.4.2	3.3.4.3
0.250%	3.3.4.4	3.1.4.3	4.2.4.3
0.125%	2.3.4.4	2.1.4.2	4.4.4.1
MS 222			
0.20%	4.4.4.4	4.3.4.3	2.2.2.2
0.10%	4.4.4.3	4.3.4.4	4.1.4.4
0.05%	4.2.4.3	4.3.4.1	4.1.4.4
PHENOXYETHANOL			
1.00%	1.-.-.-	4.3.4.4	1.-.-.-
0.50%	1.-.-.-	4.3.4.4	2.1.3.3
URETHANE			
2.0%	4.2.4.4	3.2.4.2	1.-.-.-
1.0%	4.2.4.4	3.1.4.1	3.1.4.3
0.5%	3.2.4.3	2.1.4.2	1.-.-.-
LIME TREE			
1.0%	1.-.-.-	1.-.-.-	1.-.-.-
VALERIAN			
1.0%	1.-.-.-	1.-.-.-	1.-.-.-
MENTHOL CRIST			
(*)	1.-.-.-	4.2.4.4	3.2.4.4
CLOVE OIL			
60 15 30 drops	3.4.4.4	3.4.3.3	1.-.-.-
40 10 20 drops	3.4.4.3	3.4.4.4	1.-.-.-
20 5 10 drops	3.4.4.4	3.3.4.4	1.-.-.-

Conditions and codes as in Table 3.

2% urethane. Menthol and drowning, tested in 4 ml of water, gave poor results.

Potamopyrgus jenkinsi (Table 3)

This is probably the easiest species to narcotize. Optimal results were obtained in approximately 30 h using 0.4%, 0.2%, 0.1%, 0.05% pentobarbital and 0.25% sodium pentobarbital. After repetition of these tests some specimens remained withdrawn. Sodium pentobarbital (0.125%) gave very good narcotization in 72 h, with similar success in the repetition. A third test with 100 specimens using 50 ml of 0.1% pentobarbital yielded very good results in 29 h for all specimens.

0.5% sodium pentobarbital yielded very good narcotization and good fixations I and II after 24 h.

Urethane (2%) worked well after 24 h, with good fixation I and fair fixation II; a repetition gave only fair results after 48 hours.

Pretests were made with specimens from the same locality captured in August 1990. Results were very poor using 0.5%, 0.1% and 0.2% MS 222, menthol (also at 25°C), 2% sodium pentobarbital, 4% urethane and drowning. Good and fair results of narcotization were obtained after 5 h with 1% sodium pentobarbital and 2% urethane respectively. Results of fixation in ethanol after submersion in liquid nitrogen for 10 sec were not satisfactory.

Pseudamnicola cf. *luisi* (Table 3)

Optimal results for narcotization and fixations I and II were obtained with 0.2% pentobarbital (21 h), 0.1% pentobarbital (30 h), 0.25% sodium pentobarbital (70 h) and 0.125% sodium pentobarbital (94 h). Similar results were obtained in repetitions of all these tests. A third test with 50 ml of 0.1% pentobarbital using dechlorinated tap water gave excellent results in 30 h for the remaining 88 specimens in the sample.

Good narcotization and fixations I and II were obtained after 24 h using menthol. Similar results were obtained in the repetition.

Although no repetition was carried out, 0.4% pentobarbital after 22 h was a good narcotic, yielding very good fixation I and good fixation II.

Results of narcotization and fixation were good after 52 h with 0.5% phenoxyethanol.

Horatia sturmi (Table 3)

Several methods were very good with this species. After 28 h, results were very good for narcotization and fixations I and II with 0.4%, 0.2%, 0.1% and 0.05% pentobarbital and menthol.

Results were similar after 51 h with 0.125% sodium pentobarbital. They were generally good with 0.5% and 0.25% sodium pentobarbital, 0.1% MS 222 and 1% urethane also in 51 h, with 0.5% phenoxyethanol (although the specimens seemed to have the skin removed) and 2% urethane in 44 h, and in 67 h with valerian.

Slight depigmentation was observed in black specimens after using menthol and urethane. Because no repetitions were carried out for this species, it is not possible to asses if such depigmentation was due to a direct effect of these products or to an excessive exposure to them, resulting in a slight maceration.

Good extension resulted for 54 specimens in the sample kept for 7–9 days inside the refrigerator in water from their original locality (33 fully extended, 10 good, 5 fair and 6 withdrawn).

Ancylus fluviatilis (Table 4)

Best results for narcotization and fixations I and II were obtained with 0.1% pentobarbital, 1% and 0.5% sodium pentobarbital, 2% urethane and menthol. The same results were obtained with 0.2% pentobarbital without repetition. Time of exposure was always between 4 h and 7 h.

Pentobarbital (0.4% and 0.05%), sodium pentobarbital (0.25% and 0.125%), MS 222 (0.2% and 0.1%), urethane (1% and 0.5%), valerian (1%) and 10 and 5 drops of clove oil were good or very good narcotics with an action time between 4 h and 7 h, but fixation was not as good as with the former methods. No repetitions were made.

Lymnaea peregra (Table 4)

There were difficulties experienced with fixation II. For example, after 5 h, 0.4% and 0.2% pentobarbital and 2% and 1% urethane gave very good narcosis, although specimens retracted with the fixative. However, after 76–96 h, 0.4%, 0.2%, 0.1% and 0.05% pentobarbital and 0.125% sodium pentobarbital were very successful narcotics, with very good fixation I and good fixation II, also in the repetitions. Sodium pentobarbital (0.25%) gave very good results at the same time, being only good in the second test. Two further tests were carried out using 0.1% pentobarbital and 0.125% sodium pentobarbital, the results being exceptionally good at 93 h for the first dilution (30 specimens in 100 ml of water). In the second case, narcotization was good after 136 h.

Narcotization with 1% urethane was very slow (162 h) though very good, but fixation II was only fair. Urethane 2% also gave generally good results in 94 h. Using 1% lime tree (100 h) and menthol (5 h) good results were obtained for the first tests (bad fixation II with menthol), but not for the repetition.

First tests with 10 and 5 drops of clove oil and 0.5% phenoxyethanol were good for narcotization and fixation I in 5 h, but very poor for fixation II. Repetitions of these tests yielded poor results in 72 h.

Unio sp. (Table 5)

Very good narcotization and fixations I and II were obtained after 24 h using 0.2% MS 222, although after repeating the test, fixation II was only fair. Very good results but with an action time of between 24 h and 80 h were also obtained with 0.05% and 0.1% pentobarbital, 0.1% and 0.05% MS 222 and 1% and 2% urethane. The same results were obtained in repetitions, except in the case of 0.1% pentobarbital in which narcotization

was not as successful as in the initial test. Fixation I was very good in all cases, however the animal tended to close its valves 24 h later (except with 2% and 1% urethane in which fixation II was very good).

In 72 h the test with 0.5% urethane gave good relaxation and fixations I and II, but in repetition narcotization was fair and the animal was withdrawn with valves open.

Good results were obtained with 0.2% and 0.025% pentobarbital in 24 and 72 h, respectively, 1% and 0.25% sodium pentobarbital in 24 and 30 h, respectively, and in the three tests with clove oil (in 24 h). With clove oil, a slight disturbance of the epithelium was occasionally observed.

Pretests were carried out with specimens from the Miño River collected in July 1990. Results were very good with 0.05%, 0.1% and 0.2% MS 222 after 143, 29 and 50 h, respectively. Only in the third case, valves remained open after immersion in cold ethanol. Narcotization was also very good with 30 drops of clove oil and 4% and 2% urethane in about 6 h. Subsequently, specimens were submerged in liquid nitrogen for 10 sec, and only for one sec in the case of 2% urethane. Results of fixation were good in the first and third cases, and unsatisfactory in the second.

In similar pretests with specimens from the Gasset reservoir collected in November 1989, animals were relaxed with valves open but with the foot withdrawn using 2% phenoxyethanol (29 h) and 1% and 2% sodium pentobarbital, both for 6 h. In the last two cases, valves remained open after fixation, having previously been submerged in liquid nitrogen for 10 sec. Finally, two experiments were made with 150 ml of deionized water using 1% urethane at 20°C (and posterior fixation with ethanol) and at 5°C (fixation with cold ethanol). Results of narcotization and fixation were good for the former and fair for the second, both in 10 days.

Pisidium amnicum (Table 5)

Best results of narcotization and fixations I and II were obtained with 0.5% and 1% phenoxyethanol in 30 and 48 h, respectively. Repetition of both methods was very successful in 49 h. A third test carried out with 24 specimens in 50 ml of water with 0.5% phenoxyethanol, yielded exceptionally good results in 29 h. Narcotization was excellent with 0.5% sodium pentobarbital after 49 h, but in the repetition fixation II was only fair. With 0.1% and 0.05% MS 222, narcotization was very good, time ranging between 48 and 56 h, while fixation II was unsatisfactory in both tests. Initial results of narcotization and fixations I and II with 0.1% MS 222 were very good in only one specimen in 30 h. Sodium pentobarbital (0.25%) gave good results in only one specimen, at 73 h, and also in the repetition.

Sodium pentobarbital (1%), urethane (2%), 10 drops of clove oil and menthol yielded good or very good narcosis in 24–72 h, but in the repeated tests only one of the two specimens was successfully relaxed. Good results were obtained for only one specimen using 15 drops of clove oil in 24 h. Fixation II with 2% urethane was only fair but very good with the other products and also in the repetitions. The only test made with 1% urethane gave good narcotization in 73 h, very good fixation I and a poor fixation II.

Using 0.05% pentobarbital, the minimum time neccessary to obtain good results of narcosis and fixation was 73 h.

MS 222 (0.2%) produced very good narcotization in 48 h in just one specimen, and good fixations I and II. Results were very bad for repetitions.

Results with 0.1% and 0.025% pentobarbital in 96 h and with 5 drops of clove oil in 30 h, were satisfactory.

Pretests were carried out with specimens from the same location collected in August 1990. Narcotization was excellent with 0.05%, 0.1% and 0.2% MS 222 in 5, 6 and 24 h, respectively, and with menthol (in 24 h in 4 ml of water). Fixation with ethanol was unsatisfactory in the first case and satisfactory in the others. Immersion during 10 sec in liquid nitrogen was not successful in the first case, and in the second, specimens remained open but the epithelium seemed to be broken. Sodium pentobarbital (1%) produced good narcotization in 24 h. Fixation in ethanol was also good. Results were poor with 2% sodium pentobarbital and 2% and 4% urethane, as in the test with menthol at temperature between 24°C and 28°C.

Corbicula fluminea (Table 5)

The most satisfactory narcotic was 0.1% MS 222 in 130 and 100 h, fixations I and II being excellent. The third test carried out with 49 specimens in 250 ml of solution produced excellent results in 94 h. MS 222 (0.05%) produced a very good narcosis in

100 h but fixation II was a little worse in the repetition. Pentobarbital (0.05%) in 100 h and 0.25% sodium pentobarbital in 71 h, produced very good narcotization and fixation I, and good fixation II. The same occurred in the repetition of both methods.

For 24 h, 0.125% sodium pentobarbital was a very good narcotic, with very good fixation I but poor fixation II. For 72 h, fixation II was just fair.

Menthol and 0.5% sodium pentobarbital were good narcotics after 48 h, being very good fixation I and good fixation II. When increasing action time of narcotics to 72 h, fixation II with menthol was very good, but results with 0.5% sodium pentobarbital were unsatisfactory.

Although repeated tests were not made, narcotization and fixation I were very good using 0.2% and 0.1% pentobarbital in 96 h, fixation II being only fair. Likewise without repetition, tests made with 0.4% pentobarbital (in 96 h) and 1% urethane (in 168 h) demonstrated that both were good narcotics with good and very good fixation I, respectively, and good fixation II. Using 2% and 0.5% urethane gave very unsatisfactory results.

Pretests were made with specimens from the same location collected in August 1990. The volume of dilution was between 5 and 7 ml. Narcotization and fixation were good using 0.05%, 0.1% and 0.2% MS 222 and 2% urethane in 140 h. Fixation with cold ethanol was satisfactory. One specimen, although well relaxed with 1% sodium pentobarbital in 3 h, closed its valves after fixation in ethanol. When menthol was used at a temperature between 24°C and 28°C during 50 h, the specimen was closed with the foot extended and remained so after fixation in ethanol. A similar test at 12°C was completely unsuccessful.

DISCUSSION

Among species tested in this study, pulmonate gastropods were the most susceptible to narcotization, most of the methods tried giving good results; pentobarbital, sodium pentobarbital, menthol and urethane were the most successful agents, MS 222 being good for *Ancylus fluviatilis,* phenoxyethanol and lime tree for *Lymnaea peregra,* and clove oil for both. The most universal agents for narcotizing freshwater prosobranchs were pentobarbital and sodium pentobarbital. The second most effective product was urethane (especially for *Bithynia tentaculata, Potamopyrgus jenkinsi,* and *Horatia sturmi*), followed by menthol (results were fair with *Valvata piscinalis* and with *Bithynia tentaculata* and failed with *Potamopyrgus jenkinsi*) and MS 222 (good results with *Bithynia tentaculata* and *Horatia sturmi*). Phenoxyethanol was only good for *Pseudamnicola* cf. *luisi* and *Horatia sturmi.* Regarding bivalves, MS 222, urethane, pentobarbital and sodium pentobarbital were, in this order, the best products, with phenoxyethanol being the best method for *Pisidium amnicum* and clove oil for *Unio* sp. and *Pisidium amnicum,* although ineffective for *Corbicula fluminea.*

This brief summary shows that there is a considerable variation between species in their susceptibility to narcotic agents, which is in agreement with Runham et al. (1965). This variation, which is also found between species belonging to close genera of the same group, may be observed in the degree of extension obtained and/or in the narcotic action time, and suggests that there are many factors involved in the response of freshwater molluscs to narcotization, among which can be: the physiological status of the animal (i.e. season of the year in which animal is captured, time living in aquaria), origin of the water used for dilutions, volume ratio of narcotic dilution *versus* animal, temperature and aging of narcotic dilutions.

McCraw (1958) refers to the seasonal difference of *Lymnaea stagnalis* in response to narcotic agents. We found some differences between pretests and tests in *Bithynia tentaculata* with 0.1% and 0.05% MS 222, in *Pisidium amnicum* with 2% urethane, and in *Potamopyrgus jenkinsi* with 1% sodium pentobarbital. However, these differences do not seem to be relevant since changes registered were not always coincident and because responses to the other methods tested were similar between two set of tests. This also suggests that origin of the water used for dilutions is not likely to influence results, at least for species living in changing environments. Differences between pretests and tests in *Bithynia tentaculata* may have been caused by any of these factors, although the most feasible hypothesis is that in pretests the animals died when exposed to the narcotics, because of their weakened condition after three months living in aquaria.

Corbicula fluminea differed from other species in that results of all methods varied between specimens collected in August (pretests) and in January (tests). In this species, results were contradictory as sometimes they improved (menthol) and sometimes they worsened (1% sodium pentobarbital and 2% urethane). Considering the adaptive potential of an invasive species such as *C. fluminea,* the water used for dilutions would not seem to be the cause of such differences. In this case, the observed narcotization results could be caused by physiological seasonal differences.

Similar results were obtained in repetitions of the tests in most of the species, although in some cases poorer results were registered. This was the case of *Pisidium amnicum, Potamopyrgus jenkinsi* and *Bithynia tentaculata.* Even though contradictory results might be due to the time specimens lived in aquaria, to aging of the solutions or to both, the fact that changes were not observed in the remaining species studied would point to different physiological responses of the animals to the same narcotics.

Several authors (van der Schalie, 1953; McCraw, 1958; Meier-Brook, 1976a) observed that time needed for relaxation was generally shorter for smaller animals. We found this trend for basommatophorans and prosobranchs tested, especially remarkable being the long time needed for the *Bithynia* and the *Valvata* species. Among the bivalves tested, no general rule could be observed regarding size; for instance *Unio* sp. and *Pisidium amnicum* could be relaxed, with the same narcotic and concentration, in the same time.

Giusti & Pezzoli (1980) recommend "drowning" for hydrobiids, although in our early experiments results were acceptable only with *Lymnaea peregra* and *Melanopsis* sp. Therefore, it does not seem to be useful, not only in terms of time, but also the difficulty in checking response to mechanical stimulus without disturbing the drowning process by introducing air into the jar.

The most effective narcotics for freshwater molluscs were pentobarbital and sodium pentobarbital. Both relaxed all species tested in an excellent extended position, although the optimal concentration needed varied. The minimum effective concentration of pentobarbital was 0.025% for *Unio* sp. and *Pisidium amnicum,* although 0.05% was necessary for narcotize the rest of species. The time used by this drug for the last species is the same even if the concentration is higher. The independence between drug-concentration and action time found in *Pisidium amnicum* was also observed in pulmonates and many prosobranchs. For all species tested, a concentration between 0.1% and 0.2% pentobarbital is recommended, the results using the highest concentration (0.4%) being generally worse. Overdosing with this drug seems unlikely (Meier-Brook, 1976a). Conversely, Meier-Brook (1976a) points out the risk of overdosing using sodium pentobarbital and recommends doses between 0.05% and 0.1%. We have obtained good results using higher concentrations, but beyond the limit of 0.5%, results were irregular.

Both pentobarbital and sodium pentobarbital are expensive and classified as "controlled substances," therefore not easily available, especially sodium pentobarbital. We agree with Meier-Brook (1976a), that pentobarbital is the most advisable relaxing agent for freshwater molluscs. With this product there is danger of deposits of white dust over specimens. However, this effect, due to low solubility of the product, disappears when specimens are cleaned or in the moment of fixation. According to Meier-Brook (1976a), pentobarbital has the advantage of raising the concentration gradually, avoiding shock produced in the animals by overdosing.

The effectiveness of pentobarbital and the selected concentrations were further tested by us on samples of four other different species in the following aqueous solutions: 0.1% in 8 ml was employed to narcotize two specimens of *Lymnaea truncatula* (Müller, 1774) and 30 specimens of *Neohoratia* cf. *coronadoi* (Bourguignat, 1870), 0.2% in 10 ml was used for 36 specimens of *Neohoratia schuelei* Boeters, 1981, and 0.2% in 15 ml for five specimens of *Physa acuta* Draparnaud, 1805. With all the species the results obtained were good.

Menthol has usually been employed as a narcotic for invertebrates in general and for molluscs in particular. It has been highly recommended by Berry (1943) for Amnicolidae after trying "a dozen anesthetics." From experience of two of us (M. A. R. and D. M.) with species of different genera (*Lymnaea, Physa, Ancylus, Ferrissia, Gyraulus, Planorbarius, Potamopyrgus, Pseudamnicola, Mercuria, Horatia, Neohoratia, Belgrandia, Belgrandiella,* and *Theodoxus*) successful results

were generally obtained using menthol at room temperature, especially in winter. However, results in the present experiments were not as good as expected, especially in the case of *Potamopyrgus jenkinsi.* This unpredictability has already been reported (van der Schalie, 1953; McCraw, 1958), although results may be improved transferring animals to hot formalin (Van Eeden, 1958; Runham et al., 1965) or to hot water. Probably the traditional use of this product is mainly due to the fact that it is available, cheap (also recyclable) and an easily handled product with acceptable results over a wide range of species of the different groups of freshwater molluscs.

Data in Michelson (1958) agree with our results regarding the swelling of parts of the anatomy of pulmonate snails with urethane, such swelling is easy to observe in such species as *Ancylus fluviatilis* and *Unio* sp. treated with this product. We have carried out no tests to check the further extension of narcotized specimens in contact with distilled water as occurs in pulmonates. Problems with necrosis or autoamputation (Michelson 1958) have not been detected by us. 1% urethane seemed to be the minimum effective concentration of this product for use as a narcotic for prosobranchs and for *Lymnaea peregra,* agreeing with the observation of Runham et al. (1965) on *L. stagnalis.* This inexpensive and easily available product is harmful to humans and must be handled with care.

Lime tree and valerian, though easily accessible, are not very successful narcotics. With the former, fair results may be obtained with *Melanopsis* sp., *Horatia sturmi,* and *Ancylus fluviatilis,* and good results with *Lymnaea peregra.* Valerian works fairly well with *Melanopsis* sp. and *Lymnaea peregra* and well with *Horatia sturmi* and *Ancylus fluviatilis.* Both are not advisable for bivalves. Clove oil, is a cheap, non-toxic product that gives good results with pulmonates and bivalves (except for *Corbicula fluminea*). This product and phenoxyethanol (an inexpensive but toxic product) sometimes leave deposits over the specimens which are easily cleaned.

MS 222 mixed with sodium pentobarbital was used by Joosee & Lever (1959) and Lever et al. (1964) to anaesthetize freshwater molluscs. Used for the first time as a narcotic by us, it gave excellent results in bivalves. However, as similar results can be obtained with other methods, it is not recommended because of its high price and special requirements (it must be protected from light and stored in cold).

Bad fixation obtained on *Melanopsis* sp. with diethylether after excellent relaxation suggest that it might be a good anaesthetic but not a narcotic.

While looking for a universal method for narcotize freshwater molluscs, we designed the experiences here described to standardize as much as possible the procedures for narcotization. However, if only one species or a few of them are to be studied, or available time is restricted, it is possible to improve the results. Use of mixtures of some of the agents tested have been reported to yield good results sometimes (van der Schalie, 1953; McCraw, 1958; Runham et al., 1965). It is therefore advisable to carry out specific trials before starting long-term studies. If time is a problem, then it is also important to bear in mind that the process of relaxation can be considerably shortened by carrying out narcotization at room temperature. The risk in this case can be uncontrolled death or maceration of the animals, requiring a very close monitoring.

While questions still remain, we hope we have established the choice of a useful narcotic method for each one of a wide range of species of freshwater molluscs, from a wide range of drug choices.

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LAND-SNAIL COMMUNITY MORPHOLOGIES OF THE HIGHEST-DIVERSITY SITES OF MADAGASCAR, NORTH AMERICA, AND NEW ZEALAND, WITH RECOMMENDED ALTERNATIVES TO HEIGHT-DIAMETER PLOTS

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ABSTRACT

Basic data are presented for newly reported sites (= areas of 4 ± 2 hectares) of highest known land-snail diversities for the tropics (Manombo, Madagascar [MDG]: 52 shelled species) and North America (Pine Mountain, Kentucky, U.S.A. [USA]: 42 shelled species) and are compared with Waipipi Reserve (= Jones Bush), New Zealand, the highest-diversity site known for the world (NZL: 56 shelled species, despite higher figures in the literature). MDG, with mostly new and endemic species in nine families vs. NZL's five families, belies Solem's (1984) opinion that tropical rainforests are not very diverse and adds great urgency to the need for collecting tropical land snails on the verge of extinction. Among the three sites, shell-size distributions differ conspicuously: minute species (diameter < 5 mm) are twice as dominant at NZL as at USA, with MDG intermediate; medium to large species (10–40 mm) are two to three times as prevalent at USA as at MDG, and are virtually absent at NZL; and only MDG has giant species (> 40 mm). Shell-shape distributions also differ markedly: USA and MDG are both Cainian bimodal, but with different secondary peaks at H/D 1.8 vs. 3.6, and NZL is strictly unimodal; flat-to-subglobose (H/D 0.4–0.8) is the most common shape at all three sites, but is twice as common at NZL as at MDG, with USA intermediate; only USA has very flat shells (H/D < 0.4), and only MDG has extremely tall shells (H/D > 3.2), whereas tall shells (H/D > 2.0) are entirely absent at NZL. Ecological and taxonomic differences among the three sites were used to construct simple models assuming pure natural selection and pure long-term phylogenetic constraints. Predictions of these models suggest that both natural selection and phylogenetic constraints are necessary to explain observed community morphologies, and also that additional factors, including chance colonization history and short-term phylogenetic constraints on rapidly speciating clades, played important roles. Cainian height-diameter plots compound two mathematically independent variables—size (e.g. diameter) and shape (height/diameter)—that seem better treated separately. Height and diameter, however, miss much of the relevant variation in land-snail shells, which seem better defined by the coiling aperture's rates of expansion, downward translation, and outward displacement (Raupian parameters modified for mathematical independence); a simple method is presented for calculating these three variables from five measurements taken from shell x-rays.

Key words: tropical biodiversity, biogeography, Gastropoda, community morphology, natural selection, phylogenetic constraints.

INTRODUCTION

Land-snail communities occur nearly worldwide, with sympatric species diversities ranging from one (subantarctic islands) to a predicted 72 (Waipipi Reserve [= Jones Bush], Manakau Peninsula, North Island, New Zealand), and with more than 30 species believed to be extremely rare, especially in tropical rainforests, where "snails . . . generally are neither diverse nor abundant" (Solem, 1984). Recent collections in Madagascar (Emberton, unpublished), however, have brought to light a small patch of lowland rainforest (adjacent to the village of Manombo, south of Farafangana, Fianarantsoa Province) with at least 52 sympatric, native, shell-bearing species. In addition to this unexpectedly high diversity, species at Manombo are strikingly variable in size, ranging in shell diameter from 1.2 mm to 70.4 mm. While collecting and sorting, the author was impressed by the difference in shell-size distribution of this site from others he had collected, most notably in eastern North America and in New Zealand. Standard methods for comparing land-snail community morphologies (Cain, 1977, 1978a, 1978b, 1981a, 1981b; Cameron, 1988; Cameron & Cook, 1989; Heller, 1987) seemed inadequate for

testing this impression, so alternatives were used and investigated. The purposes of this paper are (1) to provide basic data on the Madagascar site and on the most diverse site known in eastern North America (Hubricht, unpublished), (2) to compare the shell-size and shell-shape distributions of these two sites and of New Zealand's—and the world's—richest site (Solem, et al., 1981; Solem & Climo, 1985), (3) to evaluate the possible roles of natural selection and phylogenetic constraints in producing differences among these sites, and (4) to show the need for and to recommend alternative methods for comparing land-snail community morphologies.

MATERIALS AND METHODS

Sites Compared

Comparisons were made among three sites that had been collected with enough experience, care, and intensity to assure that the entire shelled malacofauna—including the minute, usually under-represented species—was sampled close to its entirety. The Manombo, Madagascar, site had been sampled once for "macros" and once for "micros." For both collections, the villagers of Manombo were given instructions on methods and search images for finding snails, and were offered good prices for all shells, with bonuses for live snails. The people of Manombo proved to be ardent collectors, many of them individually besting the author. The "macro" collection was made 27 September 1990, 10:00 am–12:00 noon, by an estimated 45 people, including the author's party, for a total of about 90 person-hours. The "micro" collection was made 16 September 1992 by 66 villagers collecting all day, for an estimated 450+ person-hours.

The area covered by the collectors is unknown, but seems unlikely to have surpassed a few hectares. Sounding the horn after the "macro" collection brought everyone walking to the off-road car within just a few minutes. Microhabitats for the "micros" were densely distributed within the forest and required long periods of collecting time, so the total area covered in a day—even by 66 people—would also probably not have been more than a few hectares. The total collecting area, judging from the fraction the author was able to see, was uniform lowland, hot-humid rainforest (Koechlin et al., 1974; Emberton, in press a) with some selective cutting but generally intact, with flat terrain, many large smooth-barked trees, many lianas and epiphytes, no outcrops or rocks, and broad-leafed litter of shallow-to-moderate depth.

Sorting to highly conservative morphospecies by the author yielded a total diversity of 52 species (Emberton, 1994a, unpublished). This is surely an underestimate, because microarboreal and subsoil habitats were undercollected and because both visits were during the dry season, but should give an adequate picture of shell-size and -shape distributions. This (52 species) seems to be the highest diversity report for a tropical land-snail collection site; the second highest is a rainforest station in New Caledonia, where repeated collections over the course of a year by expert collectors yielded 41 species (Tillier, 1989a).

According to Leslie Hubricht (in litt.), who is the most experienced living collector of land snails of the eastern United States (Hubricht, 1985), that region's most diverse site is a small area (presumably less than four hectares) on Pine Mountain, Harlan County, Kentucky, which has yielded 44 land gastropods, of which 42 are shelled snails and 2 are slugs (Hubricht, unpublished). This diversity is unsurpassed by any known site in either the western United States (Barry Roth, pers. comm.), Mexico (Fred Thompson, pers. comm.), or Canada (Pilsbry, 1939–1945; Cameron, 1988), so it is the richest known in North America. (The previous North American record was 41 gastropod species, taken from a larger area: Solem, 1984.)

Hubricht visited the Pine Mountain site during the spring of at least four different years, each time collecting intensively with the express purpose of finding as many species as possible (Hubricht, pers. comm.). The author and John Petranka made an intensive, productive collection within 2 km of Hubricht's site on 9 May 1982. The area has a dense, old-second-growth, mixed hardwood forest on a limestone base, with rich soil, a deep leaf-litter cover, and an uneven ground with many large rocks.

New Zealand's Waipipi Scenic Reserve (= Jones Bush, southwest of Auckland), a small (4.2 ha), remnant, partially degraded patch of temperate rainforest, was collected 3 January 1977 by David Roscoe and Bruce Hazlewood, and 10–14 and 17 February 1981 by Frank Climo, David Roscoe, and the late Alan

Solem (Solem et al., 1981). The long experience and skill of these collectors, the thoroughness of their methods (including litter sieving and flotation), and their primary objective of getting all species, combined to assure an accurate representation of the true malacofauna. In total, the site yielded 56 native shelled snails and at least one native slug (Solem et al., 1981: 462, appendix 3A). This number indicates a lower diversity than cited elsewhere for Waipipi Reserve (Jones Bush): "about 72 native species is a probable reality" (Solem et al., 1981: 453), "exceeds 70 species" (Solem, 1984: 12), "60 species [of native land snails and slugs] have been collected [in a 2 hectare patch]" (Solem & Climo, 1985: 1). Nevertheless, Waipipi Reserve remains the most diverse known site in the world, and its number of known species would probably be increased by additional collecting (Solem et al., 1981).

The terrain, vegetation, and snail microhabitats of Waipipi Reserve were described and partially illustrated by Solem et al., (1981). The present author collected with Climo and Roscoe in a similar patch of bush on the northwest South Island of New Zealand in June 1984. These patches lie within steep gulleys (hence their escape from wood-cutters, fires, and sheep-and cattle-grazers) and have a variety of trees forming a dense canopy, no outcrops or rocks, and a generally very deep and diverse leaf litter that includes curled fronds of palms and fern trees.

Thus all three sites have been well sampled for all size categories of land snails by experienced collectors. Exact sampling areas are unknown but all seem to be on the order of 4 ± 2 hectares.

Shell-Size Comparisons and Predictions

For the Madagascan (MDG) and North American (USA) sites, an "average" shell of each species was measured for height and diameter using vernier calipers or an ocular micrometer on a dissecting microscope. No measurement data were readily available for the New Zealand site (NZL), but Solem & Climo (1985: table 2) had published shell-diameter distributions of 83 species of the Manakau Peninsula, given in 0.5 mm intervals. After subtracting the four introduced species, the diameter distribution of the remaining 79 species was taken as indicative of that of NZL's 56 species (= a subset of the 79).

Shell diameter was used as an index of shell size. This index is advantageous for its simplicity, its ease of interpretation, and its ecological relevance in approximating the minimum-diameter opening through which a snail can carry its shell into shelter (see below). Because this index is so approximate, because shell size can vary tremendously within a single population of land snails (Goodfriend, 1986; Emberton, 1988a), and because NZL's size distribution is represented by an inflated number of species, shell size is treated in this paper in only very broad categories. Other indices that have been used for shell size—approximate volume (Solem et al., 1981) and height-plus-diameter (Gould, 1984)—also have their shortcomings; it seems unlikely that using either of these alternative indices would significantly change the results of this analysis. Shell-size histograms were based on the diameter intervals of 0.50–5.00 mm (minute), 5.01–10.00 mm (small), 10.01–20.00 mm (medium), 20.01–40.00 mm (large), and 40.01+ mm (giant).

To simplify size distributions for modeling, the small and large size classes were deleted. Thus predictions were made for three disjunct size classes: minute (< 5 mm), medium (10–20 mm), and giant (> 40 mm).

Predictions based on pure natural selection assumed that available size niches are filled with no constraints on selection other than those imposed by long-term climatic conditions. Each of the three sites was scored on a scale of one to three for its possession of physical niches (whether filled or not) of the three sizes minute, medium, and giant, and for its climatic aids toward filling those niches by long-term freedom from frost, drought, and severe storms. The importance of each niche-filling aid to each size category of snails was then ranked from one to three. The resulting tables were then used to predict the representation of each snail size at each site. For example, to calculate the predicted micros at USA, the USA frost-free score was multiplied by the importance of frost-freedom to micros, and this product was added to the USA drought-free score times the importance of drought-freedom to micros, plus the USA storm-free score times the importance of storm-freedom to micros; the resulting sum was then multiplied by USA's score for its number of physical niches

for micros. From the resulting table of predictions, a histogram of predicted shell-size distributions was prepared for each site, scaled for direct comparison with the histogram of actual shell-size distributions.

Predictions based on pure phylogenetic constraints assumed that (a) each family is no more size-constrained in these three sites than it is throughout its total world distribution, and (b) within each site, species will vary randomly within their family's world-wide size range, unrestricted by natural selection. Families (and groups of related families) representing 10% or more of the species at any of the three sites were arranged phylogenetically, following Nordsieck (1986; see Emberton & Tillier, 1994, regarding Tillier, 1989b). Each of these families (or groups of related families) was categorized by its total range of shell diameters (simplified as minute, medium, and giant), both within the three sites and worldwide. Worldwide family size ranges were determined using the collections of the Academy of Natural Sciences of Philadelphia, guided by Zilch (1959–1960). For each site, the number of species falling into minute, medium, and giant size classes was counted for each of the dominant families (or groups of related families). This number was then divided equally among the size classes occupied by the family worldwide. The site's predicted number of species in each size class was obtained by adding such results over all dominant families. A histogram of these predictions was prepared, scaled for direct comparisons with the site's actual size distributions.

Shell-Shape Comparisons and Predictions

Shell height/diameter (H/D) was used as an index of shell shape (Cain, 1977). For the Madagascan (MDG) and North American (USA) sites, H/D was calculated for the "average" shell of each species from height and diameter measurements described above. To represent the New Zealand site (NZL), H/D values for 77 native species of the Manakau Peninsula (including Waipipi Reserve = NZL) were taken from Solem & Climo (1985: fig. 6). Comparative histograms used ten intervals: H/D = 0.00–0.40 (very flat), 0.41–0.80 (flat to subglobose), 0.81–1.20 (globose), 1.21–1.60 (moderately elevated), 1.61–2.00 (elevated), 2.01–2.40 (moderately tall), 2.41–2.80 (tall), 2.81–3.20 (very tall), 3.21–3.60 and 3.61–4.00 (both extremely tall).

To simplify shell-shape distributions for modeling, four disjunct categories were used: very flat (H/D < 0.41), globose (H/D = 0.81–1.20), tall (H/D = 2.01–2.80), and extremely tall (H/D > 3.21). Natural-selection predictions were based on the demonstrated tendencies for flat-shelled and tall-shelled species to forage on horizontal and vertical surfaces respectively, with globose-shelled species variable and more versatile (Cain & Cowie, 1978; Cameron, 1978, 1981; Cook & Jaffar, 1984; Heller, 1987); in addition, it was assumed that very flat shells are effective for escaping drought in narrow crevices. Each of the three sites was scored on a scale of one to three for its possession of physical niches (inclination angles of smooth surfaces; narrow, unflooded crevices) for the four shape categories. The importance of each of three niche-filling aids (long-term freedom from frost, drought, and severe storms: see above) to each of these shell-shape categories was ranked from one to three. Predictions of the representation of each snail shape at each site followed the methods described above for shell size.

Phylogenetic-constraints predictions for shell shape used the same methods described above for shell size, except that if any species in a dominant family at a site fell within one of the four shape categories, then all the site's members of that family were included in the computations.

Orthogonal Raupian Parameters

Raup's (1961, 1966) W, D, and T (= the coiling aperture's rates of expansion, outward displacement, and downward translation) are mathematically correlated when calculated from the periphery of the aperture (Emberton, 1986, 1994b). Several different modifications of Raupian methods, however, have made W, D, and T orthogonal by taking the geometric center of the aperture as the standard point of reference (Raup & Graus, 1972; Harasewych, 1982; Illert, 1983; Okamoto, 1984, 1988). To demonstrate the advantages of orthogonal W, D, and T over height and diameter, two tall shells and two flat shells of very different ontogenies but identical height/diameters were sketched in cross-section, based on recent experience with shell x-rays (Emberton, in press, in prep.), and an easy method was devised for calculating W, D, and T. The four shells were then plotted for comparisons in two different

morphospaces: Cainian (height vs. diameter) and Raupian (W vs. D vs. T).

RESULTS

Site Data

Appendices 1–3 list the species of MDG, USA (with permission of L. Hubricht), and NZL in systematic order, with shell measurements given for MDG and USA. Systematics studies of MDG are in progress (Emberton, 1994a, unpublished), so the species, most of which are new, are simply numbered within tentative genera. All of Hubricht's collections from USA and the author's collections from near that site (author's station GS-119) are at the Field Museum of Natural History, Chicago.

Shell-Size Comparisons

Figure 1 compares the shell-size distributions of USA, MDG, and NZL. Minute species (shell diameter 0–5 mm) are everywhere a major component, but contribute less than half as strongly in USA (40%) as in NZL (84%), with MDG intermediate (63%). Small species (5–10 mm) are roughly equivalent throughout: NZL 15%, MDG 13%, USA 22%. However, medium (10–20 mm) and large (20–40 mm) species are three times and two times as common in USA (24%, 14%) as in MDG (8%, 8%), and are virtually absent in NZL (0%, 1%). Among the three sites, only MDG has giant species (40–70 mm), which comprise 8% of its diversity.

Selection-Based Size Predictions

Table 1 shows the size-class model and its predictions assuming pure natural selection. Availability of minute physical niches (whether filled or not) was scored intermediate for USA (deep, broad-leaf litter; crevices in rocks, logs, and rough-barked tree trunks) and MDG (shallow, broad-leaf litter; crevices in logs, palm-tree trunks, and numerous epiphytes and vines), but high for NZL (extremely deep and complex litter; crevices in logs and palm-tree trunks). Physical niches for medium snails were scored intermediate at all sites, with the lack of rock shelters at MDG and NZL compensated for by fallen palm boles. Availability of giant physical niches seems to depend on ease of mobility among rare sheltering large logs (present at all sites), so was scored low for NZL (with dense, loose, rough-surfaced litter obstructing movement), intermediate for USA (with a fairly smooth litter surface but many rough stones and rough-barked trees and logs), and high for MDG (smooth surfaces throughout, including most trees and logs).

The climatic aids to filling these niches differs among sites. Freedom from frost is highest at tropical MDG, intermediate at maritime-temperate NZL, and lowest at continental-temperate USA. Freedom from drought is highest at NZL (nearly regular rainfall augmented by temperate-coast fogs and streamside topographic shelter), intermediate at MDG (short dry season under tropical sun), and lowest at USA (occasionally rainless summers, intensified by rapid drainage through the limestone base). Freedom from the effects of major storms was scored highest at NZL (in a well-sheltered gulley), intermediate at USA (exposed mountainside, thunderstorms and occasional blizzards and hailstorms), and lowest at MDG (exposed to fairly frequent cyclones and with yearly torrential rains).

The importance of these climatic factors toward niche-filling ability by land snails depends on their size category. Frost is a greater obstacle to giants than to minutes (which can more easily escape into deep, narrow crevices and, because of their faster thaw times, can more easily evolve physiological adaptations to body freezing), with mediums intermediate. Drought, on the other hand, more rapidly and drastically affects minutes than giants (which with their lower surface-to-volume ratio can withstand desiccation longer, and with their greater mobility can better seek saving shelter), with mediums intermediate. Severe storms not only knock snails from their physical niches, but also transport them, causing gene flow that can thwart natural selection adapting them to local niches. These effects are strongest on minute snails, weakest on giants, and intermediate on mediums.

Table 1 summarizes these scores. Resulting predictions of shell size classes at each site are also given in Table 1 (bottom).

Phylogeny-Based Size Predictions

Nine families (or groups of related families) contributed at least 10% of the species to at least one of the three sites; together they

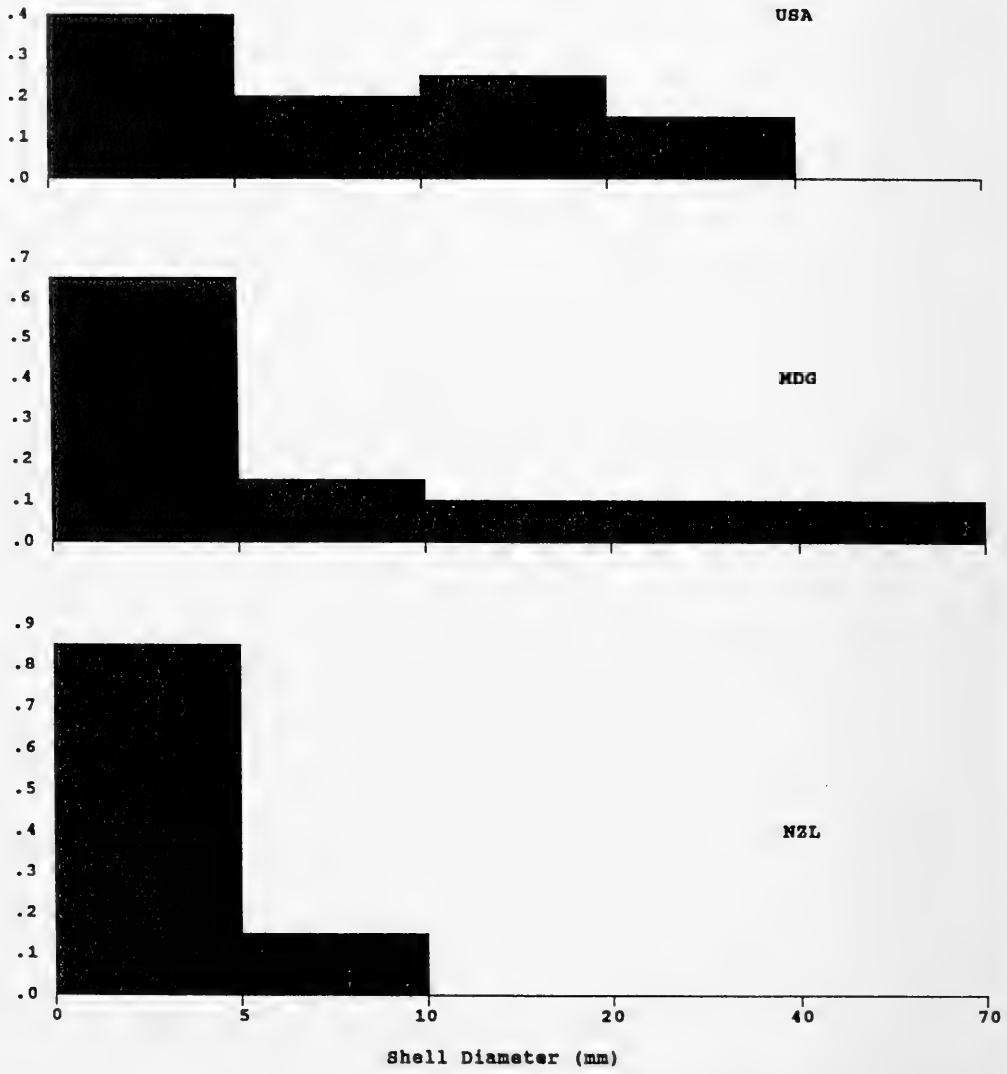


FIG. 1. Shell-size distributions of the native land snail species in the most diverse known sites of North America (USA), Madagascar (MDG), and New Zealand (NZL). The vertical scale is the proportion of total species, rounded to 0.05.

comprised 90%, 96%, and 84% of the total species diversities of MDG, NZL, and USA, respectively. Figure 2 lists these dominant families (or groups of related families) phylogenetically, arranged top to bottom from most ancient (= plesiomorphic = "primitive") to most recent (= apomorphic = "derived").

Figure 2 also arranges the three sites according to the overall phylogenetic age of their faunas. Of the three, MDG has the strongest representation of ancient taxa, including most of the prosobranchs (Cyclophoridae), all of the presumably more ancient families of the Achatinida (*sensu* Nordsieck, 1986), and most of the presumably more ancient families of the most recent Helicida. NZL is next, with its complement of prosobranchs, its dominance by Achatinida, and its absence of Helicida; and USA follows, with all of the most recent Helicida (but also with the "primitive" Vertiginidae). As in other geographically isolated land-snail faunas (Cain, 1977, 1978a, 1980; Peake, 1978; Cameron & Cook, 1992), phylogenetic overlap among these

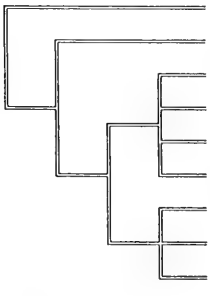
TABLE 1. A model to predict shell size distributions assuming pure natural selection

Site	Physical Niches			Niche-Filling Aid		
	Minute	Medium	Giant	Frost-free	Drought-free	Storm-free
USA	2	2	2	1	1	2
MDG	2	2	3	3	2	1
NZL	3	2	1	2	3	3

Niche-Filling Aid	Importance to Snails		
	Minutes	Mediums	Giants
Frost-free	1	2	3
Drought-free	3	2	1
Storm-free	3	2	1

Site	Predicted Snails		
	Minutes	Mediums	Giants
USA	20	16	12
MDG	24	24	36
NZL	60	32	12

USA = Pine Mountain, Kentucky, U.S.A.; MDG = Manombo, Fianarantsao Province, Madagascar; NZL = Waipipi Reserve (= Jones Bush), North Island, New Zealand. Physical-niche scores: 1 = rare, 2 = intermediate, 3 = common. Scores for historical presence of niche-filling aids: 1 = rare, 2 = intermediate, 3 = common. Scores for importance of niche-filling aids to size classes of snails: 1 = low, 2 = intermediate, 3 = high. See text for method of calculating predicted size-class representations. Shell size classes: minutes = 0–5 mm diameter, mediums = 10–20 mm, giants = 40–70 mm.



	% of Species			Shell Size		Shell Shape	
	MDG	NZL	USA	These Sites	World-Wide	These Sites	World-Wide
Cyclophor-Liarei	29	4	0	min-med	min-med	glob-tall	vflat-xtall
Vertiginidae	0	0	14	min	min	tall	glob-tall
Subulinidae	12	0	0	min	min-med	tall-xtall	tall-xtall**
Streptaxidae	10	0	0	min	min-med	tall-xtall	vflat-xtall
Acavidae	10	0	0	gnt	med-gnt	glob	vflat-tall
Punct-Charop-Disc	4	92	10	min-med	min-med	vflat-glob	vflat-tall
Helixarion-Eucon	25	0	5	min-med	min-med	glob	glob**
Zonitidae	0	0	29	min-med	min-med*	vflat-glob	vflat-glob
Polygyridae	0	0	26	med	med*	(flat)	vflat-glob
Total	90	96	84				

FIG. 2. Evolutionary relationships, sizes, and shapes of the superfamilies dominating the most diverse land snail communities of Madagascar (MDG), New Zealand (NZL), and North America (USA). The evolutionary tree follows Nordsieck (1986). Abbreviated family names: Cyclophoridae and Liareidae; Punctidae, Charopidae, and Discidae; Helixarionidae and Euconulidae. Shell sizes: min = minute = diameter 0–5 mm; med = medium = 10–20 mm; gnt = giant = 40–70 mm. Shell shapes: vflat = very flat = height/diameter (h/d) 0.0–0.4; (flat) = h/d 0.4–0.8; glob = globose = h/d 0.8–1.2; tall = h/d 2.0–2.8; xtall = extremely tall = h/d 3.2–4.0. * very rarely and only barely reaches giant size (Zonitidae: *Aegopis, Poecilozonites;* Polygyridae: *Neohelix major*). ** excluding the systematically problematic, very-flat-shelled *Cupulella* (?Subulinidae) and *Roybellia* (?Helixarionidae).

three sites is minimal, with no species or genera and extremely few families in common (Appendices 1–3).

In addition, Figure 2 gives the shell-size categories covered by these nine dominant families (or groups of related families), both within the three sites and worldwide. Size ranges are the same except in Subulinidae,

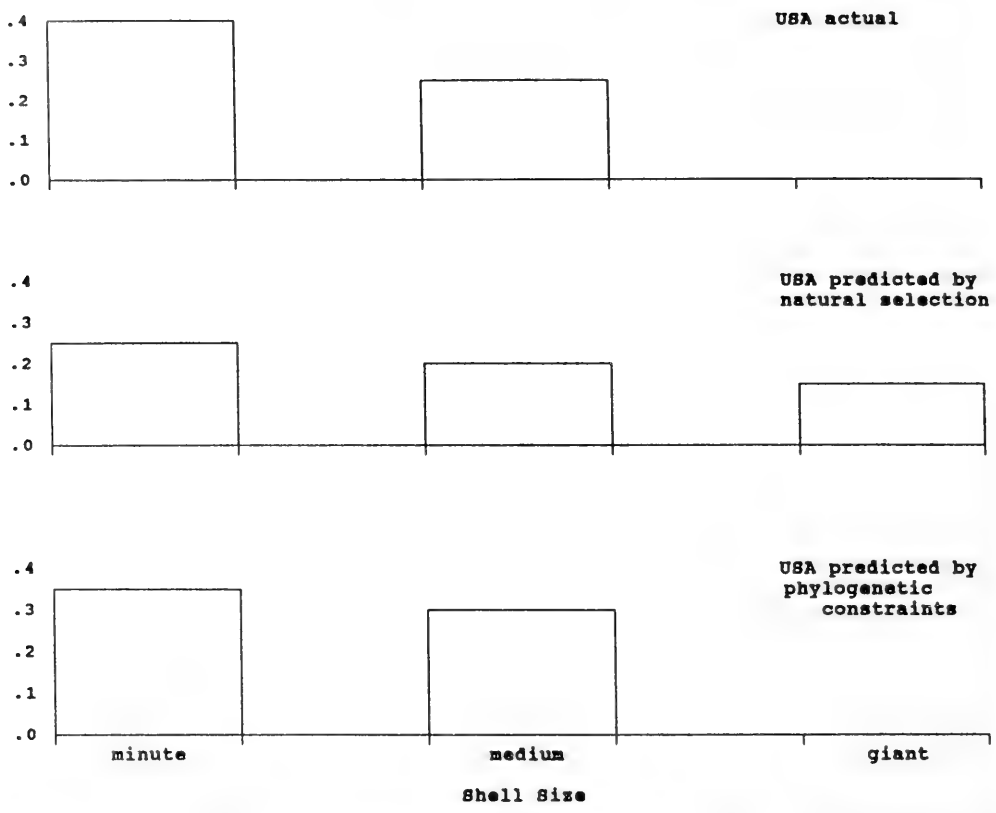


FIG. 3. Shell-size distributions at Hubricht's (unpublished) site on Pine Mountain, Kentucky, U.S.A., as recorded and as predicted by models assuming pure natural selection (Table 1) and pure phylogenetic constraints (see text).

Streptaxidae, and Acavidae *sensu lato* (Emberton, 1990), which do not reach medium size at the three sites but do elsewhere.

Numbers of species from the dominant families (and groups of related families) falling into minute, medium, and giant size categories (from data in Appendices 1 and 2, and Solem & Climo, 1985: table 2) were: MDG 38, NZL (actually the Manukau Peninsula; see Methods) 64, and USA 20. Redistributing these species among size categories under the assumption of phylogenetic constraints free from natural selection yielded for MDG 17 minute, 19 medium, and 2 giant; for NZL 32 minute, 32 medium, and 0 giant; and for USA 10.5 minute, 9.5 medium, and 0 giant.

Actual vs. Predicted Size Distributions

Figures 3–5 compare distributions of minute, medium, and giant species as actually sampled (top), as predicted from pure natural selection (middle), and as predicted from pure phylogenetic constraints (bottom). For USA (Fig. 3), both selection and phylogeny were adequate predictors of minute and medium categories, but only phylogeny correctly predicted the absence of giant species.

For MDG (Fig. 4), selection predicted more giants than mediums, and phylogeny predicted more mediums than giants, neither of them reflecting reality in themselves, but in combination predicting the natural equality between mediums and giants (Fig. 4: top). Neither predictor by itself or in combination, however, could account for the high natural representation of minute species.

For NZL (Fig. 5), phylogeny, but not selection, accurately predicted the absence of giants; and selection, but not phylogeny, accurately predicted the predominance of minutes. Neither predictor, however, accounted for the absence of mediums.

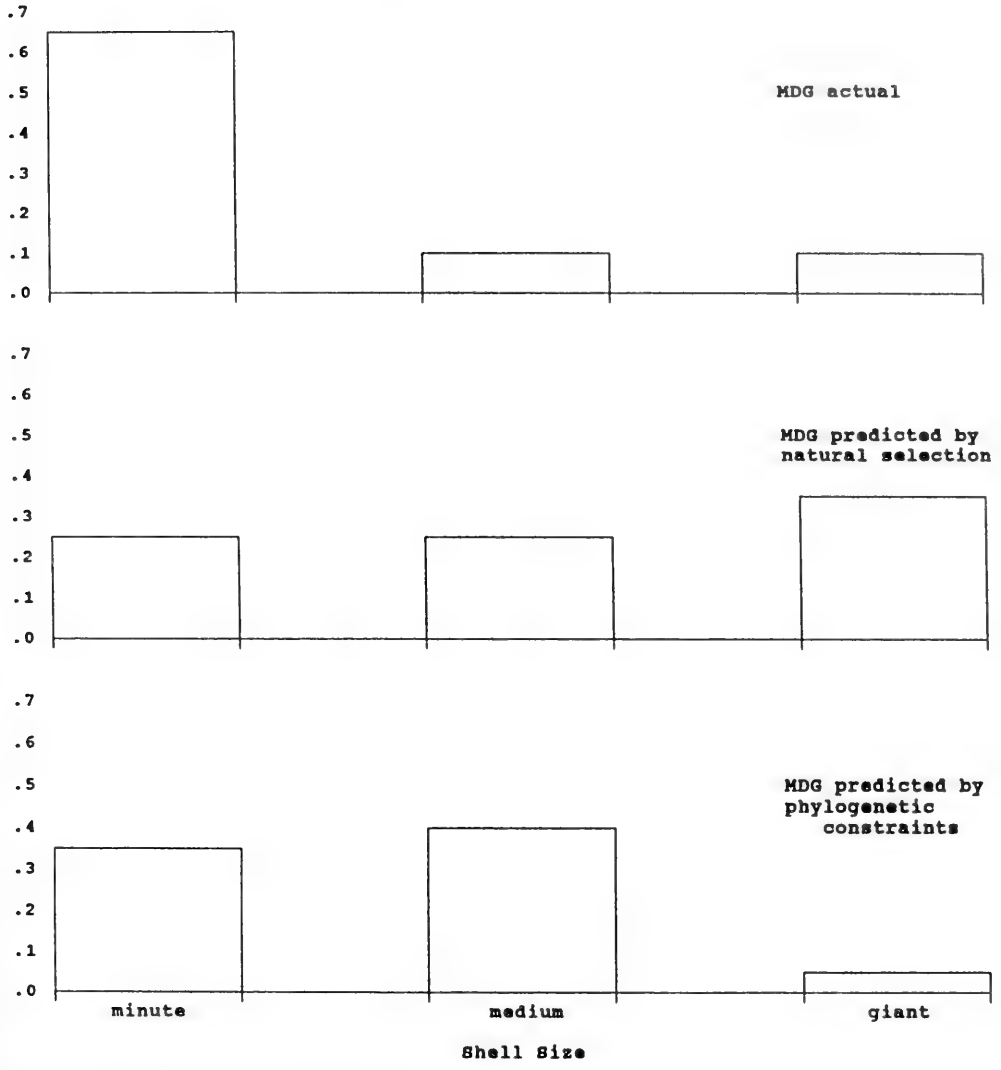


FIG. 4. Shell-size distributions at the site near Manombo, Fianarantsao Province, Madagascar, as recorded and as predicted by models assuming pure natural selection (Table 1) and pure phylogenetic constraints (see text).

Shell-Shape Comparisons

Figure 6 compares shell-shape distributions among the three sites. USA and MDG are both Cainian bimodal with a primary peak at about H/D 0.6, but with the secondary peak at about H/D 1.8 for USA and about H/D 3.6 for MDG. NZL, in contrast, is strictly unimodal. Flat-to-subglobose (H/D 0.4–0.8) is the most common shape at all three sites, but is 2.5 times as common at NZL (83%) as at MDG (33%), with USA intermediate (64%); only USA has very flat shells (H/D < 0.4), and only MDG has extremely tall shells (H/D > 3.2), whereas tall shells (H/D > 2.0) are entirely absent at NZL.

Selection-Based Shape Predictions

Table 2 shows the pure-selection model and its predicted shape-class distribution for each site. Very flat physical niches (whether filled or not) were scored common at USA

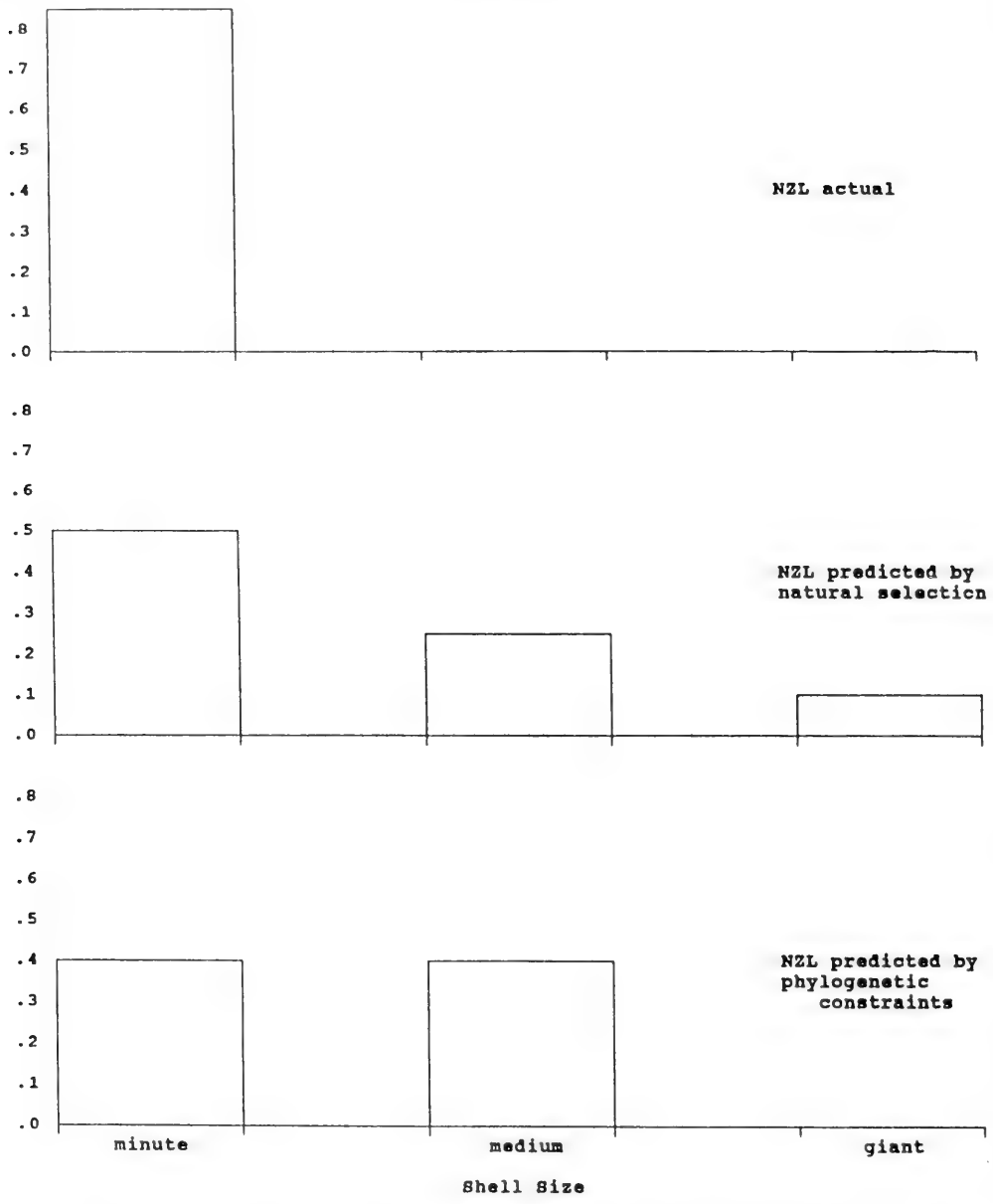


FIG. 5. Shell-size distributions at Waipipi Reserve (= Jones Bush), North Island, New Zealand, as represented by Manakau-Peninsula snails (Solem & Climo, 1985) and as predicted by models assuming pure natural selection (Table 1) and pure phylogenetic constraints (see text).

(within rock crevices, under loose bark of logs, and under rocks) but rare at both MDG and NZL, where rocks are few, log bark is less detachable, and other narrow crevices (e.g. at the bases of palm-tree branches) are usually too wet to provide shelter. Niches for globose snails were scored intermediate at all sites, due to their varieties of inclinations of crawling surfaces: rocks, logs, and trees at USA; logs, trees, and epiphytes at MDG; and trees and deep, complex litter at NZL. Vertical foraging niches for both tall- and ex-

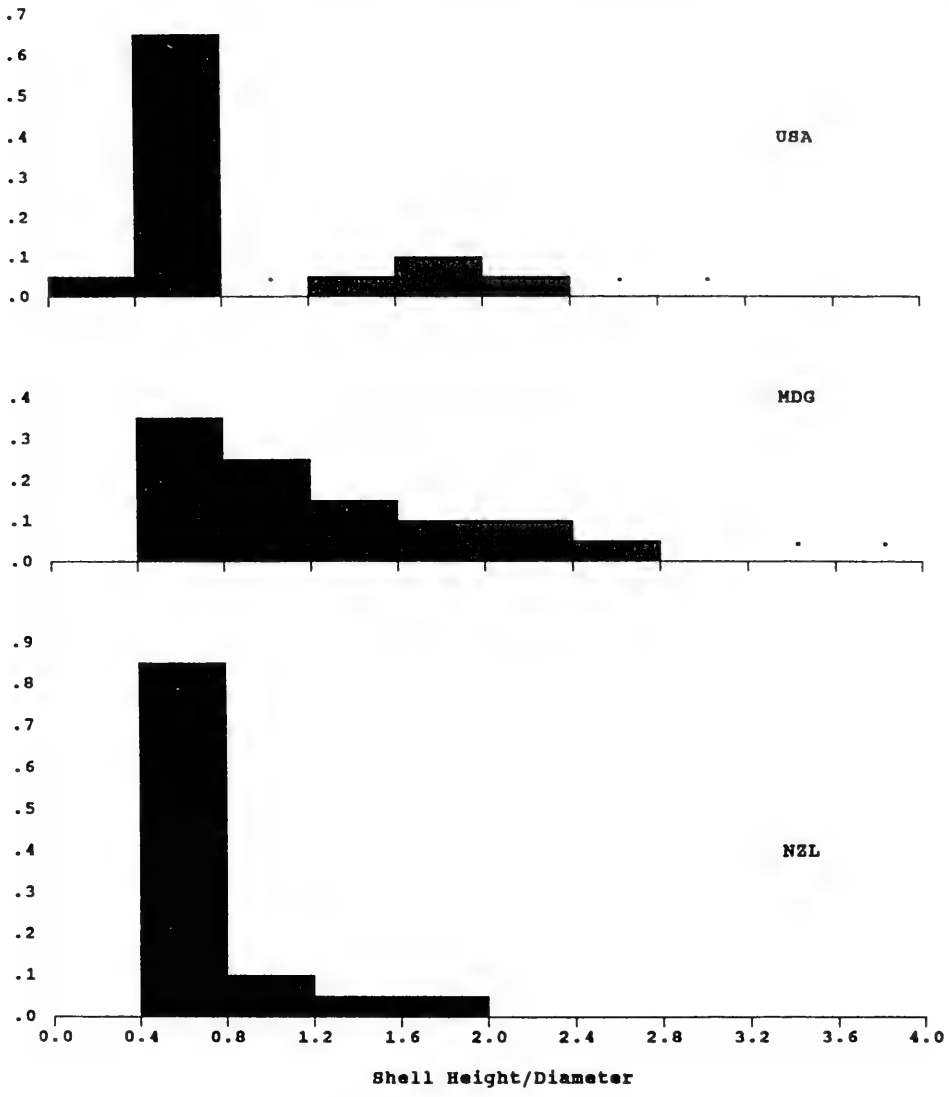


FIG. 6. Shell-shape distributions of the native land snail species in the most diverse known communities of North America (USA), Madagascar (MDG), and New Zealand (NZL). Dots signify non-zero proportions < 0.025.

tremely tall-shelled snails were scored common at MDG (many smooth-barked trees and big, broad, smooth leaves; smooth-surfaced litter allowing migration), rare at NZL (few smooth-barked trees; deep, uneven-surfaced litter blocking migration), and intermediate at USA (few smooth-barked trees, but smooth-surfaced litter).

Long-term climatic aids to filling these niches were scored previously (Table 1). The importance of these climatic factors toward niche-filling ability by land snails varies according to shell shape. Although very flat-shelled snails can enter narrow, deep refuges to escape frost, drought, and major storms; tall- and extremely tall-shelled arboreal snails are openly exposed to (and are highly vulnerable to) these threats, with globose-shelled snails intermediate.

Table 2 summarizes these scores and uses

TABLE 2. A model to predict shell shape distributions assuming pure natural selection

	Physical Niches			
	VFlat	Globose	Tall	XTall
Site				
USA	3	2	2	2
MDG	1	2	3	3
NZL	1	2	1	1
	Importance to Snails			
Niche-Filling Aid				
Frost-free	1	2	3	3
Drought-free	1	2	3	3
Storm-free	1	2	3	3
	Predicted Snails			
Site				
USA	12	16	24	24
MDG	6	24	54	54
NZL	8	24	24	24

Sites and scores are as in Table 1. See text for method of calculating predicted shape-class representations. Shell shape classes: vflat = very flat = height/diameter (h/d) 0.0–0.4, globose = h/d 0.8–1.2, tall = h/d 2.0–2.8, xtall = extremely tall = h/d 3.2–4.0.

them to predict shell-shape distributions for the three sites.

Phylogeny-Based Shape Predictions

Figure 2 shows shell-shape ranges covered by the nine dominant families (or groups of related families), both within the three sites and world-wide. Shape ranges are generally greater worldwide, with only subulinid, helixarionid-euconulid, and zonitid worldwide ranges fully covered in the three sites.

Redistributing all species of the nine dominant families (or groups of related families) among shape categories under the assumption of phylogenetic constraints free of natural selection yielded for MDG 7.33 very flat, 20.33 globose, 10.33 tall, and 8.00 extremely tall; for NZL 16.75 very flat, 16.75 globose, 16.75 tall, and 0.75 extremely tall; and for USA 12.8 very flat, 17.8 globose, 4.3 tall, and 0.0 extremely tall.

Actual vs. Predicted Shape Distributions

For USA (Fig. 7), natural selection was a better predictor of the tall-shell category, but only phylogenetic constraints predicted the absence of extremely tall shells. Although both these factors predicted the presence of very flat and globose shells, neither predicted the natural predominance of very flat over globose shells.

For MDG (Fig. 8), both factors successfully predicted the presence of tall, extremely tall, and globose shells, but phylogenetic constraints was more accurate in predicting the natural ratio of globose to tall plus extremely tall. Furthermore, the natural-selection model's success in these categories was entirely accidental, because all of the tall and very tall MDG species were not arboreals as predicted, but ground dwellers (*Hainesia* sp. 1, "*Subulina*" sp. 2, "*Edentulina*" spp. 1, 2, *Streptostele* sp. 1), and all of MDG's known arboreals were not tall as predicted, but subglobose to globose (*Tropidophora* spp. 1–3, *Ampelita* sp. 2, *Helicophanta* sp. 2, *Kaliella* sp. 1). Neither the natural-selection nor the phylogenetic-constraints model was able to predict the absence of very flat species from MDG.

For NZL (Fig. 9), natural selection was better at predicting the dominance of the globose shells, and phylogenetic constraints was better at predicting the absence of extremely tall shells. Neither factor, however, succeeded in predicting the complete absence from NZL of both tall and very flat shells.

Orthogonal W, D, and T vs. Height and Diameter

Figure 10 compares two tall shells and two flat shells. The shells are hypothetical but biologically plausible, with similarities to actual living species (Zilch, 1959–1960). The shells

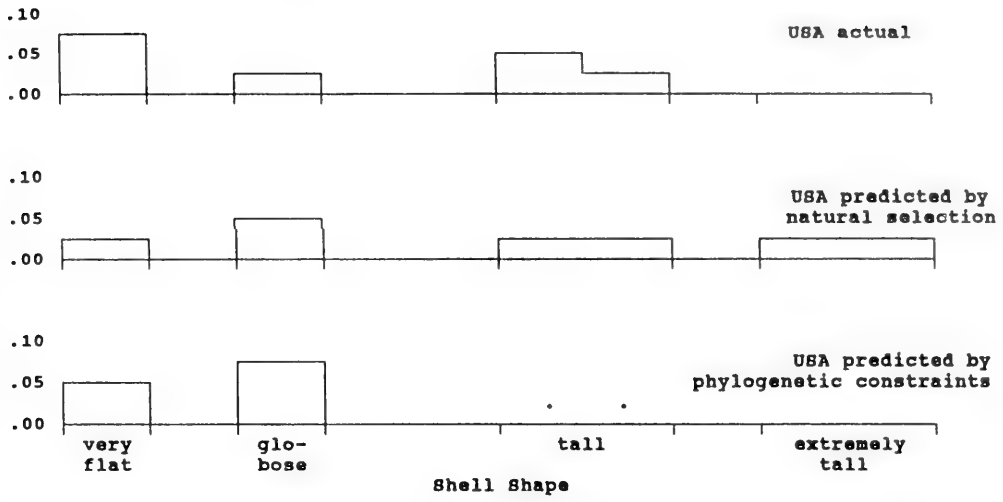


FIG. 7. Shell-shape distributions at Hubricht's (unpublished) site on Pine Mountain, Kentucky, U.S.A., as recorded and as predicted by models assuming pure natural selection (Table 2) and pure phylogenetic constraints (see text). Dots signify non-zero proportions < 0.0125.

are shown in cross section as they would appear in an x-ray (Hutchinson, 1989: fig. 3; Emberton, 1994a). The two standard measurements traditionally used in land-snail community morphology (Cain, 1977, ff.; this paper) are: height and diameter = maximum shell dimensions parallel to and perpendicular to the shell's central axis of rotation (Fig. 10b).

Alternative measurements needed for calculating mathematically independent versions of Raupian parameters (Raup, 1961, 1966) are also shown in Figure 10. Two apertures w whorls apart are matched in area by circles with the same geometric centers (in Fig. 10 these circles are estimated by eye, but they could be generated more precisely by a computerized algorithm). Four measurements are then taken from the circles' centers: r_1 and r_2 = radii of the smaller and larger circles (Fig. 10d), and d and t = distances between the circles' centers perpendicular to and parallel to the shell's axis of rotation (Figs. 10a, c). Orthogonal coiling parameters are calculated:

$$W = (\pi/w)\ (r_2^2 - r_1^2)$$

$$D = d/w$$

$$T = t/w$$

Table 3 provides all these measurements and calculated variables for Figure 10's four shells.

Figure 10e plots the four shells in Cainian two-dimensional morphospace Cain (1977, ff.). In this height-diameter plot, shells a and b occupy the same point in the upper (tall-shelled) region, and shells c and d occupy the same point in the lower (flat-shelled) region. Alternative size and shape plots (not figured) as used in this paper would show single values for a and b at smaller and taller positions on the unidimensional scale line than the single values for c and d.

Figure 10f plots the same four shells in the recommended alternative, Raupian three-dimensional morphospace (Raup, 1961, 1966). In this plot, each shell occupies a unique position, and the tall and flat shells b and c are closer to each other than either is to the other tall or flat shell a or d.

DISCUSSION

Tropical Land-Snail Diversity and Extinction

The most important message of this paper is the urgent need to collect disappearing tropical land-snail faunas as quickly as possible. Manombo Reserve, Madagascar (MDG), contains the tropics' most diverse known site, which is close to and may eventually surpass Waipipi Reserve, New Zealand (NZL), as the world's most diverse known

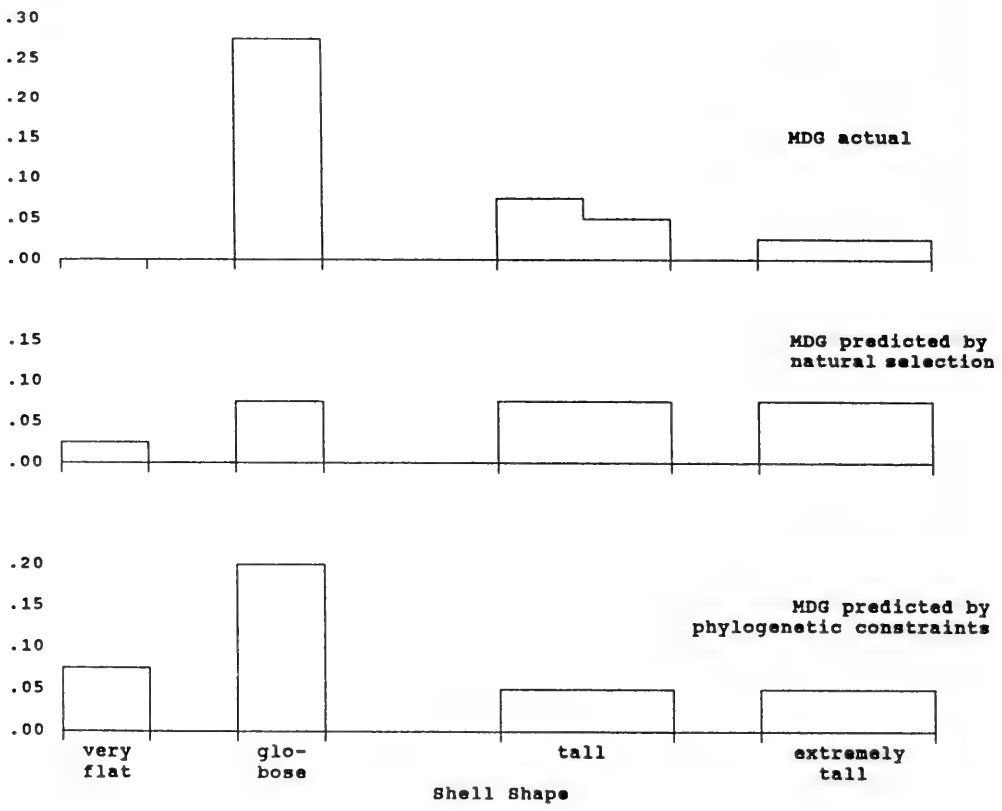


FIG. 8. Shell-shape distributions at the site near Manombo, Fianarantsao Province, Madagascar, as recorded and as predicted by models assuming pure natural selection (Table 2) and pure phylogenetic constraints (see text). Dots signify non-zero proportions < 0.0125.

site. MDG, with mostly new and endemic species in nine—mostly phylogenetically ancient—families vs. NZL's five families (Appendices 1, 3), might even already rank higher than NZL in molluscan *genetic* diversity. These data, added to accumulating evidence of high land-snail diversities at rainforest sites in Peru (R. Ramírez, pers. comm.), Costa Rica (C. Altaba, pers. comm.), mainland Africa (De Winter, 1992), and New Caledonia (Tillier, 1989a), refute Solem's (1984: 17) unsubstantiated claim that in tropical rain forests "snails . . . generally are neither diverse nor abundant."

The fact is that although tropical rain-forest snails generally are not abundant (thus requiring very intensive collecting), they can often be extremely diverse. Vast regions of the tropics are uncollected or undercollected for land snails (Solem, 1984). Such high sympatric diversities, coupled with the patterns of tiny geographic ranges, high degrees of endemism, and extreme ecological fragilities well known in land snails (Tillier & Clarke, 1983; Solem, 1984, 1990; Murray et al., 1988; Emberton, 1994a, in press a), means that no system of parks and reserves, no matter how extensive, can prevent major extinctions during the next few decades. Not only must a large fraction of tropical land-snail biodiversity lie outside of reserves, but reserve status is no guarantee of protection (Soulé, 1991). Manombo Reserve is a good case in point, with a village in its midst, and with deforestation still actively taking place in May 1993 (personal observations).

Besides the importance of collecting sheer numbers of disappearing tropical species, emphasis needs to be placed on oceanic islands as refugia for ancient clades that have already undergone some extinction on continents. The two islands in this study, Madagascar and New Zealand, show this trend beautifully (Fig. 2). The additional tendency of

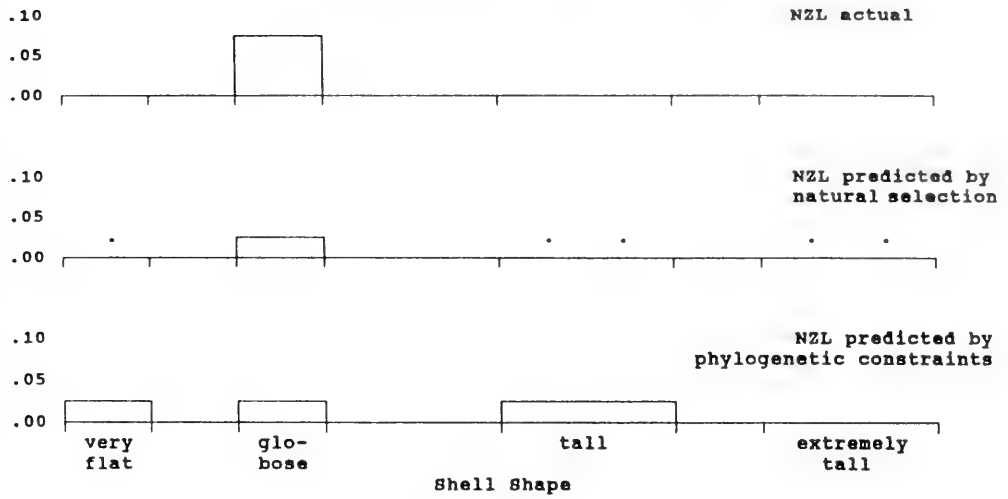


FIG. 9. Shell-shape distributions at Waipipi Reserve (= Jones Bush), North Island, New Zealand, as represented by Manakau-Peninsula snails (Solem & Climo, 1985) and as predicted by models assuming pure natural selection (Table 2) and pure phylogenetic constraints (see text). Dots signify non-zero proportions < 0.0125.

oceanic islands to have a phylogenetically depauperate fauna (Peake, 1978; Cameron & Cook, 1992) is obvious in New Zealand but only scarcely applies to Madagascar, the world's fourth largest island (Appendices 1, 3).

Comparing Diversities

For land-snail biodiversity, the lines are quite indistinct between alpha-, beta-, and gamma-diversities (Cameron, 1992; = sympatric, mosaic, and allopatric diversities of Solem, 1984: 11). Certainly, to say that all the snails found within a four-hectare area of forest are living in sympatry would be misleading. Most Waipipi-Reserve species, for example, are well segregated by microhabitat (Solem & Climo, 1985); thus the 56-species figure for this site (Appendix 3) mixes alpha- and beta-diversity. There has yet to be a standardized sampling method that allows truly accurate biogeographic comparisons of land-snail diversities. Land snails have such high gamma-diversity (Solem, 1984), however, that the most efficient tropical collecting requires frequent movement among sites, so that standardized, intensive quadrat sampling seems counterproductive. Land snails are so patchily distributed (beta-diversity) that the best way to collect a tropical site quantitatively is to spend set amounts of time (a) searching for micros in the right microhabitats, (b) beating vegetation over inverted umbrellas for arboreal micros, (c) scanning the ground and digging out refuges for macros, and (d) scanning the trees for arboreal macros. Others disagree, and get diverse collections from ground-litter quadrats augmented by macro searches (R. Ramírez, pers. commun.) or from bagged litter samples (E. Naranjo Garcia, pers. comm.).

Another problem in comparing land-snail diversities is that of differing species concepts, especially with regard to gamma-diversity. Land-snail species are notoriously variable in shell morphology (Goodfriend, 1986) and sometimes in genitalia as well (Tillier, 1989a). Polytypic species are common, and their sometimes exuberant overdescriptions by past taxonomists are only beginning to be cleared up using modern species concepts (e.g. Gould & Woodruff, 1986). The three sites compared in this paper, however, suffer from little if any such overdescription (Hubricht, 1985; Solem et al., 1981; Emberton, 1994a).

Comparing and Predicting Community Morphologies

Height-diameter plots could have been used to compare community morphologies of USA, MDG, and NZL, but such plots com-

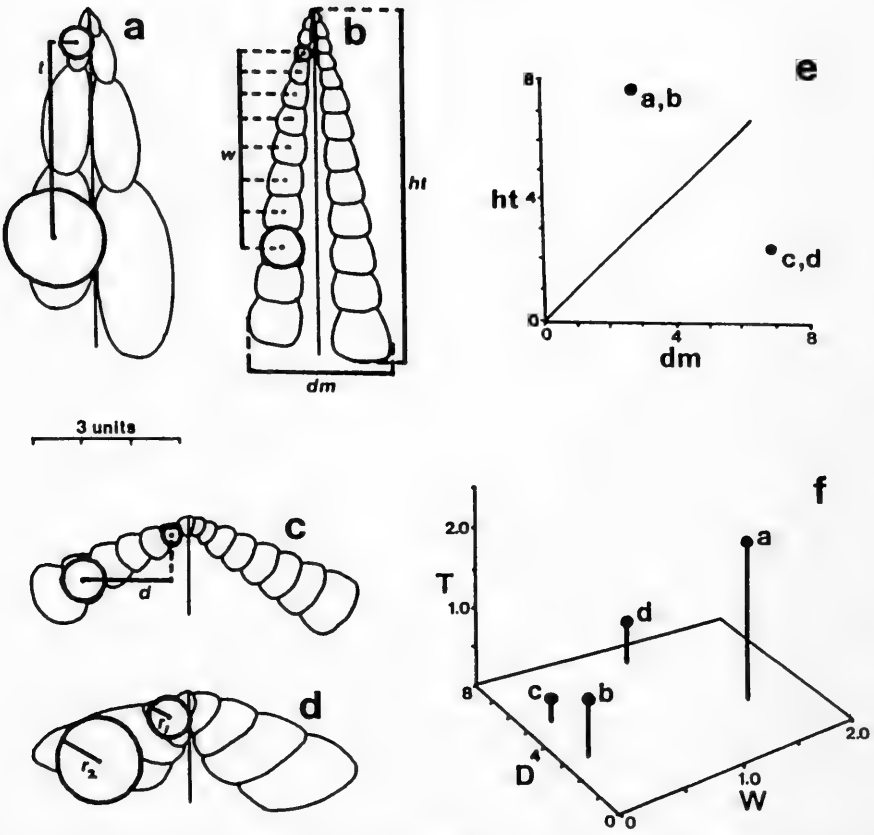


FIG. 10. Four hypothetical shells (a-d, shown as simplified x-rays) and their positions in Cainian (e) and Raupian (f) morphospaces. Cainian measurements (= parameters): dm = diameter, ht = height; Raupian measurements (taken from circles whose areas and centers coincide with apertural cross-sections): w = whorl count between circles, r_1 and r_2 = radii of circles, d and t = distances between circles' centers perpendicular to and parallel to the axis of rotation; Raupian parameters: the coiling aperture's per-whorl rates of whorl expansion ($W = [\pi/w] [r_2^2 — r_1^2]$), outward displacement (D = d/w), and downward translation (T = t/w). Table 3 lists all measurements and variables for the four shells.

pound two mathematically independent variables—size (e.g. diameter) and shape (height/diameter)—that seem better treated separately. Thus important differences among the three sites were detected in both size (Fig. 1) and shape (Fig. 6). The sites also differ enormously in ecology (Tables 1, 2) and in phylogenetic composition (Fig. 2); predictions from simple models based on these differences (Figs. 3–5, 7–9) suggest that both natural selection and phylogenetic constraints are necessary to explain observed distributions of both shell size and shape, but that even these two factors in combination are not always sufficient. Additional factors, therefore, must be involved.

A complete set of factors controlling community morphology should include proximate natural selection for both foraging-surface incline (Cain, 1977, ff.) and shelter site (Solem & Climo, 1985; this paper), climatic exclusion (Gould, 1970), long-term phylogenetic constraints (this paper), chance colonization history (Cameron, 1988; Cameron & Cook, 1992), speciation within clades with short-term phylogenetic constraints (Cameron & Cook, 1992), interspecific competition (Cain, 1977, ff.), direct environmental induction (Gould, 1970), and constructional constraints. Of these, climatic exclusion, chance colonization history, and short-term phylogenetic constraints on rapidly speciating clades seem to have played important roles in forming the community-morphology differences

TABLE 3. Measurements and calculated variables for the four hypothetical shell x-rays shown in Fig. 10

	Shell			
	a	b	c	d
Cainian				
measurement: ht.	7.5	7.5	2.3	2.3
measurement: diam.	3.0	3.0	7.0	7.0
calculation: ht./diam.	2.5	2.5	0.3	0.3
Raupian				
measurement: w	2	7	4	2
measurement: r_1	0.3	0.15	0.2	0.4
measurement: r_2	1.0	0.45	0.4	0.9
measurement: d	0.5	0.5	1.8	1.4
measurement: t	4.2	4.1	1.0	1.0
calculation: W	1.4	0.1	0.1	1.0
calculation: D	0.3	0.1	0.5	0.7
calculation: T	2.1	0.6	0.3	0.5

among USA, MDG, and NZL. Climatic exclusion may explain better than available niche space (Table 1) the absence in eastern North America of giant snails, which seem to be restricted to the tropics (Zilch, 1959–1960) and which (family Camaenidae) occupied northern North America during its tropical phase in the Cretaceous and early Tertiary (Solem, 1978). Chance alone may have prevented the extremely tall-shelled clausiliids from colonizing eastern North America from ecologically similar western Europe, thus explaining the absence of very tall shells at USA. Extensive radiations of New Zealand punctids and charopids and Madagascan cyclophorids within phylogenetically constrained genera may partially explain NZL's and MDG's dominance by minute, flat-to-subglobose shells. The absence from NZL of medium-sized snails may be due to chance colonization history, because the introduced, medium-sized *Bradybaena similaris* has found a niche there (Solem et al., 1981). Climatic exclusion, on the other hand, may have prevented the giant-shelled, New Zealand genus *Paryphanta* (Powell, 1979) from establishing at NZL. It may also be climatic exclusion that explains the absence of very flat shells from both MDG and NZL, where all narrow crevices may be too wet to serve as niches for land snails. The absence of tall and very tall shells at NZL is probably due partly to climatic exclusion (since tall charopids are found elsewhere in New Zealand) and to chance colonization history (no subulinids, clausiliids, etc., invaded the islands).

One can hope eventually to quantify the relative contributions of all these (and perhaps other) factors toward any given land-snail community morphology, in a way analagous to such efforts on individual morphology (Raup, 1972; Cheverud, 1982; Emberton, in press b). In striving toward such a goal, it seems essential to alter Cain's (1977, ff.) protocol in two ways. First, individual, highly localized communities should be the standard units of comparison, rather than regional, whole-island, or continental faunas. There is as yet no standard definition of a land-snail community in terms of collecting area. A community could be defined (and measured) as the two- or three-dimensional area within which all land-snail individuals are capable of physical contact with all (or 90% or 80% of) other species during an average generation.

Second, community-morphology comparisons should include all land molluscs. The cited works have generally treated prosobranchs separately from pulmonates, on the argument that prosobranch radulae are fundamentally so different that they must occupy different feeding niches. There seems to be little hard evidence for this, and there is considerable evidence that pulmonate radulae have adapted to a full range of niches, from the "prosobranch niche" (Cain, 1977) of scraping algal films from tree trunks (*Achatinella, Liguus*) to snagging large living prey (*Euglandina*). To try to isolate components of the land-snail fauna based on feeding niche, therefore, seems unavoidably complex and artificial, especially since there are no data on most species of most faunas. Thus fixed size and shape differences between pulmonates and non-pumonates can be attributed to

long-term phylogenetic constraints but should not be segregated *a priori.*

Gould (1970) established a valuable approach toward determining the causes of differences in community morphology when he analysed a "naturally replicated experiment" in land-snail community structure that controlled for the factors of natural selection, phylogenetic constraints, colonization, and speciation. In that study of fossil land-snail assemblages of Bermuda, Gould (1970: fig. 10) discovered major effects from climatic exclusion and measurable effects from environmental induction.

Despite such progress, the goal of "a science of form" (Gould, 1971; Raup, 1972) for land-snail community morphology still seems a long way off, because so little is known about any of the controlling factors. For example, although natural selection for taller shells to feed on more vertical surfaces seems reasonably well documented (Cain & Cowie, 1978; Cameron, 1978, 1981; Cook & Jaffar, 1984; Emberton, in press b), there is also a second adaptive shape for vertical-surface feeding: flat-shelled rock-cliff foragers that shelter in narrow rock crevices (Emberton, 1986, 1988b, 1991a; Heller, 1987). At MDG the tall-shelled snails are not vertical-surface feeders, but are restricted to the ground. Also at MDG, the arboreal species—like those of other rainforests in the Philippines and northern Australasia (Cain, 1978b)—are globose rather than high-spired. These few examples demonstrate how much remains to be discovered about natural selection on the functional morphology of shell shape. Evaluating phylogenetic constraints on land-snail shells requires robust phylogenetic hypotheses, which unfortunately are almost entirely lacking above the subfamilial level (Emberton et al., 1990; Emberton, 1991b; Bieler, 1993; Emberton & Tillier, 1994).

Cain's (1977) call for more ecological data on land-snail species remains strongly in effect, especially for tropical faunas that are rapidly going extinct. Cameron & Cook (1989) set excellent standards for rapidly evaluating ecological differences within a community. Even when such studies are not possible (e.g. during fast-moving, labor-intensive tropical surveys), recording the positions and activity states of collected specimens would provide valuable new data.

Much of the recent progress in land-snail community morphology has been due to the simplicity and elegance of height-diameter plots and the naturally bimodal patterns they detect (Cain, 1977, ff.). Height and diameter are correlated, however, and finer discrimination among patterns can be made by treating size and shape separately (Figs. 1, 6).

A much higher refinement can be achieved at the cost of x-raying (Emberton, 1994b) and taking five measurements per shell (Fig. 10b–d). Mapping community morphologies by Raup's W, D, and T (Fig. 10f) offers much more than simply adding a third dimension: these three variables, which can be further customized (Harasewych, 1982; Kohn & Riggs, 1975; Okomoto, 1988; Ackerly, 1989; Emberton & Chapman, unpublished), elegantly define much of the shell's ontogeny (but see Gould, 1968; and Hutchinson, 1989, 1990), can yield reasonably precise calculations of shell volume and surface area (Raup & Graus, 1972; in contrast to approximations by Solem & Climo, 1985), and are mathematically orthogonal, so can be analyzed independently for controlling factors (Emberton, in press b, this paper) or can be used to define a natural three-dimensional morphospace with realized regions bounded by constructional constraints, competitive exclusion, etc. (Raup, 1966).

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APPENDIX

APPENDIX 1. Manombo Reserve, Madagascar: taxonomy and shell dimensions of native shelled land snails. Introduced *Achatina* is omitted. Higher classification follows Abbott & Boss (1989) for subclasses Prosobranchia and Gymnomorpha and Nordsieck (1986) for subclass Pulmonata: order Stylommatophora.

Genus & Higher Classification	Species Number	Measurements (mm) Height	Diam.	Ht./Diam.
Subclass PROSOBRANCHIA				
Order MESOGASTROPODA				
Superfamily CYCLOPHOROIDEA				
Cyclophoridae				
Boucardicus	1	9.1	8.8	1.03
	2	5.3	5.0	1.06
	3	2.9	3.2	0.91
	4	3.2	2.9	1.10
	5	3.8	2.3	1.65
	6	2.8	2.5	1.12
	7	3.0	2.9	1.03
	8	2.5	1.6	1.56
	9	2.3	2.0	1.15
	10	3.5	2.9	1.21
	11	1.6	1.2	1.33
	12	1.9	1.2	1.58
	13	3.1	2.7	1.15
Cyathopoma	1	2.0	2.2	0.91
Hainesia	1	31.0	13.5	2.30
Superfamily LITTORINOIDEA				
Pomatiasidae				
Tropidophora	1	16.0	16.0	1.00
	2	30.0	29.3	1.02
	3	24.0	29.0	0.83
Superfamily RISSOIDEA				
Assimineidae				
Omphalotropis	1	3.9	2.9	1.34
	2	5.6	3.7	1.51
Subclass PULMONATA: Order STYLOMMATOPHORA				
Superfamily BULIMINOIDEA				
Buliminidae (= Enidae)				
Rachis	1	11.4	7.5	1.52
Suborder SIGMURETHRA				
Infraorder ACHATINIDA				
Superfamily ACHATINOIDEA				
Subulinidae				
"*Subulina*"	1	3.5	1.8	1.94
	2	2.7	1.3	2.08
	3	4.9	1.8	2.72
	4	2.9	1.2	2.42
	5	5.1	2.0	2.55
	6	13.6	3.7	3.68
Superfamily STREPTAXOIDEA				
Streptaxidae: Streptaxinae				
"*Edentulina*"	1	7.2	3.4	2.12
	2	5.2	2.3	2.26
	3	4.8	2.4	2.00
Gulella	1	2.9	1.5	1.93
Streptaxidae: Enneinae				
Streptostele	1	25.2	7.1	3.55

(*continued*)

APPENDIX 1. (*Continued*)

Genus & Higher Classification	Species Number	Measurements (mm) Height	Diam.	Ht./Diam.
Superfamily ACAVOIDEA				
Acavidae				
Ampelita	1	19.0	46.0	0.41
	2	17.3	34.5	0.50
Helicophanta	1	32.5	57.0	0.57
	2	46.7	70.4	0.66
	3	80.0	65.0	1.23
Superfamily PUNCTOIDEA				
Charopidae				
Pilula	1	5.7	9.0	0.63
	2	4.3	8.5	0.51
Infraorder HELICIDA				
Superfamily HELIXARIONOIDEA				
Helixarionidae: Sesarinae				
Kaliella	1	3.6	3.2	1.12
Helixarionidae: Microcystinae				
Microcystis	1	3.6	6.7	0.54
	2	1.7	2.3	0.74
	3	1.6	2.3	0.70
	4	2.3	3.6	0.64
	5	2.4	3.9	0.62
Helixarionidae: Ariophantinae				
Kalidos	1	9.3	17.4	0.53
	2	13.0	26.2	0.50
	3	11.0	18.0	0.61
Malagarion	1	5.6	8.3	0.68
	2	2.3	3.4	0.68
Helixarionidae: Macrochlamydinae				
Sitala	1	4.1	3.7	1.11
	2	3.3	4.8	0.69

APPENDIX 2. Pine Mountain, Kentucky, U.S.A.: taxonomy and shell dimensions of native shelled land snails. Two species of native philomycid slugs also occur: *Philomycus venustus* and *Pallifera secreta.* Classification as in Appendix 1, except that Riedel (1980) was followed for the Zonitidae. Polygyrid measurements were from Pine Mountain specimens, all others were from the literature for geographically and ecologically relevant specimens.

Genus & Higher Classification	Species	Measurements (mm) Height	Diam.	Ht./Diam.
Subclass PROSOBRANCHIA				
Order ARCHAEOGASTROPODA				
Superfamily HELICINOIDEA				
Helicinidae				
Hendersonia	*occulta*	4.0	6.6	0.61
Order MESOGASTROPODA				
Superfamily RISSOIDEA				
Hydrobiidae				
Pomatiopsis	*lapidaria*	6.3	3.2	1.97
Subclass PULMONATA: Order ARCHAEOPULMONATA				
Suborder ELLOBIOIDEA				
Ellobiidae				
Carychium	*clappi*	1.9	0.8	2.38
C.	*nannodes*	1.4	0.6	2.33
Subclass PULMONATA: Order STYLOMMATOPHORA				
Suborder ORTHURETHRA				
Superfamily COCHLICOPOIDEA				
Cochlicopidae				
Cionella	*morseana*	7.1	2.3	3.09

APPENDIX 2 (*Continued*)

Genus & Higher Classification	Species	Measurements (mm) Height	Diam.	Ht./Diam.
Superfamily PUPILLOIDEA				
Vertiginidae				
Vertigo	*gouldi*	1.6	1.0	1.60
V.	*clappi*	1.5	0.8	1.88
Columella	*simplex*	2.2	1.4	1.57
Gastrocopta	*pentodon*	1.8	1.1	1.64
G.	*contracta*	2.5	1.4	1.79
G.	*corticaria*	2.5	1.0	2.50
Suborder SIGMURETHRA				
Infraorder ACHATINIDA				
Superfamily RHYTIDOIDEA				
Haplotrematidae				
Haplotrema	*concavum*	9.0	21.0	0.43
Superfamily PUNCTOIDEA				
Punctidae				
Punctum	*blandianum*	0.7	1.2	0.58
Discidae				
Discus	*patulus*	4.0	8.9	0.45
D.	*nigrimontanus*	2.4	7.4	0.32
Anguispira	*mordax*	6.0	18.0	0.33
Infraorder ELASMOGNATHA				
Superfamily SUCCINEOIDEA				
Succineidae				
Succinea	*ovalis*	25.0	13.5	1.85
Infraorder HELICIDA				
Superfamily HELIXARIONOIDEA				
Euconulidae				
Euconulus	*fulvus*	2.4	3.1	0.77
Guppya	*sterkii*	0.8	1.2	0.67
Superfamily VITRINOIDEA				
Zonitidae: Gastrodontinae				
Gastrodonta	*interna*	5.0	7.4	0.68
Ventridens	*collisella*	7.2	8.7	0.83
Striatura	*meridionalis*	1.0	1.7	0.59
Zonitidae: Zonitinae: Vitreini				
Paravitrea	*multidentata*	2.2	4.0	0.55
P.	*subtilis*	1.4	2.9	0.48
P.	*capsella*	3.0	5.6	0.54
Zonitidae: Zonitinae: Zonitini				
Mesomphix	*inornatus*	9.8	20.0	0.49
M.	*perlaevis*	12.2	20.0	0.61
M.	*cupreus*	14.4	28.4	0.51
Vitrinizonites	*latissimus*	7.1	11.9	0.60
Glyphyalinia	*cumberlandiana*	1.2	3.0	0.40
G.	*rimula*	3.4	7.7	0.44
Superfamily POLYGYROIDEA				
Polygyridae: Triodopsinae: Triodopsini				
Neohelix	*albolabris*	21.5	31.7	0.68
Xolotrema	*denotata*	8.8	16.3	0.54
Triodopsis	*tridentata*	9.6	15.4	0.62
T.	*vulgata*	8.3	14.3	0.58
Polygyridae: Polygyrinae				
Allogona	*profunda*	15.0	28.6	0.52
Stenotrema	*edvardsi*	5.6	7.8	0.72
S.	*stenotrema*	7.1	9.9	0.72
Polygyridae: Polygyrinae: Mesodontini				
Patera	*appressa*	8.7	16.4	0.53
Inflectarius	*inflectus*	6.6	10.9	0.61
Mesodon	*zaletus*	20.2	29.2	0.69
Appalachina	*sayana*	19.0	24.2	0.78

APPENDIX 3. Waipipi Reserve (= Jones Bush), New Zealand: taxonomy of native shelled land snails. The slug *Athoracophorus bitentaculatus* is not included. Classification as in Appendix 1.

Genus & Higher Classification	Species
Subclass PROSOBRANCHIA	
Order ARCHAEOGASTROPODA	
Superfamily HYDROCENOIDEA	
Hydrocenidae	
Georissa	*purchasi*
Order MESOGASTROPODA	
Superfamily CYCLOPHOROIDEA	
Liareidae	
Liarea	*hochsteteri*
Cytora	*cytora*
C.	*torquilla*
Subclass PULMONATA: Order STYLOMMATOPHORA	
Suborder ORTHURETHRA	
Superfamily ACHATINELLOIDEA	
Achatinellidae	
Lamellidea	*novoseelandica*
Suborder SIGMURETHRA	
Infraorder ACHATINIDA	
Superfamily RHYTIDOIDEA	
Rhytididae	
Delos	*coresia*
D.	*jeffreysiana*
Rhytida	*greenwoodi*
Superfamily PUNCTOIDEA	
Charopidae	
Caviella	*buccinella*
C.	*roseveari**
Mocella	*eta*
M.	aff. *maculata*
"*Charopa*"	*pseudanguicula*
"*C.*"	*chrysaugeia*
"*C.*"	aff. *pseudanguicula* 1
"*C.*"	*fuscosa*
"*C.*"	*pilsbryi*
Fectola	*mira*
F.	*unidentata*
F.	*infecta*
Huanodon	*pseudoleiodon*
H.	*hectori*
Allodiscus	*urquharti*
A.	aff. *granum*
Geminoropa	*cookiana*
Serpho	*kivi*
Flammulina	*perdita*
F.	*chiron*
"*Thalassohelix*"	*ziczac*
Suteria	*ide*
Phenacohelix	*giveni*
P.	*pilula*
P.	n. sp. 1
Therasiella	*neozelandica*
T.	*serrata*
T.	aff. *neozelandica*
T.	*celinda*
Punctidae	
"*Laoma*"	*mariae*
"*L.*"	*marina**
"*L.*"	aff. *marina* 1
"*L.*"	*leimonias*
"*Phrixgnathus*"	*erigone*
"*P.*"	*ariel*
"*P.*"	*elaiodes*
"*P.*"	*moellendorffi*
"*P.*"	*conella*
"*P.*"	n. sp. 59
"*P.*"	*poecilosticta*
"*Paralaoma*"	n. sp. 38
"*P.*"	n. sp. 29
"*P.*"	*lateumbilicata*
"*P.*"	n. sp. 1
"*P.*"	n. sp. 8
"*P.*"	aff. n. sp. 33
"*P.*"	n. sp. 40*
"*P.*"	*serratocostata*

* collected by Roscoe and Hazlewood in 1977 but not included in Appendix 3A of Solem et al., (1981: 462).

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DISTRIBUTIONAL DIFFERENCES AMONG ACAVID LAND SNAILS AROUND ANTALAHA, MADAGASCAR: INFERRED CAUSES AND DANGERS OF EXTINCTION

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ABSTRACT

Seven species of giant, acavid land snails occur in the region of Antalaha, Diego Suarez Province, northeastern Madagascar. Analysis of 4,446 specimens collected from 39 stations along three coast-to-inland transects in October 1990 revealed significant distributional patterns in five of the species. *Clavator moreleti* was more abundant at low elevations and away from the coast, and *Ampelita xystera* occurred at an intermediate distance from the coast. Both these species were virtually absent from the southern transect (Ankavia River valley), where *A. julii* was at its most abundant. *Ampelita fulgurata* was restricted to the inland middle transect (Ankavanana River valley), and *A. soulaiana* to intermediate-inland sites on the northern transect (Andempona River valley). In contrast, neither *A. lamarei* nor *Helicophanta amphibulima* showed significant spatial heterogeneity, although the latter tended to be scarcer near the coast. Combining these data with older, published records supported the results and suggested that the distributional patterns can be explained by historical founding events (*Clavator moreleti* southward along the coast and *Ampelita julii* northward), by climatic exclusion and coastal land clearing (*Ampelita xystera*), and by local speciation events (*A. soulaiana* and possibly *A. fulgurata*). The Antalaha area is undergoing rapid forest clearing, definitely endangering *A. soulaiana* and possibly endangering *A. julii, A. fulgurata,* and the genetically distinct local race of *A. xystera.*

Key words: Gastropoda, Pulmonata, Stylommatophora, Acavoidea, rainforest biogeography, speciation, endangered species.

INTRODUCTION

This paper is the fourth in a long-term study on the phylogeny, morphological evolution, and biogeography of the acavoid land snails worldwide, beginning with Madagascan taxa because of that island's status as an environmental hotspot (Myers, 1988; Emberton, in review a). The first paper (Emberton, 1990) reviewed the acavoids as a monophyletic clade with a Gondwanan distribution and with an unusually great range of shell shapes—from globose to high-spired—wherever they occur, collated all taxonomic and distributional data on Madagascan acavids, and performed a cladistic analysis of 21 species of Madagascan acavids based on five anatomical characters from the publications of Fischer-Piette and colleagues (see Emberton, 1990, for references). The second paper (Emberton, in review b) presented a more rigorous cladistic analysis of 18 species of Madagascan acavids in the genera *Helicophanta, Ampelita,* and *Clavator* based on a new data set of 71 informative allozyme characters, and predicted from preliminary dissections that acavid terminal genitalia will help resolve their phylogeny. The third paper (Emberton, in press) analyzed the estivation sites and external body and shell morphologies of nine diverse acavid species in an evolutionary context. This paper analyzes the distributional patterns of the seven acavid species that occur in the rainforest region of Antalaha, northeastern Madagascar, and compares the results with 20-year-old data from the same region (Fischer-Piette et al., 1973) to hypothesize causes for these distributions and to assess the danger of extinction for each species.

MATERIALS AND METHODS

Collecting stations are mapped in Figure 1. Transects were run along roads and paths that followed approximately the valleys of the Andempona, Ankavanana, and Ankavia rivers, in the vicinity of Antalaha, Diego Suarez Province, Madagascar. Collecting was dur-

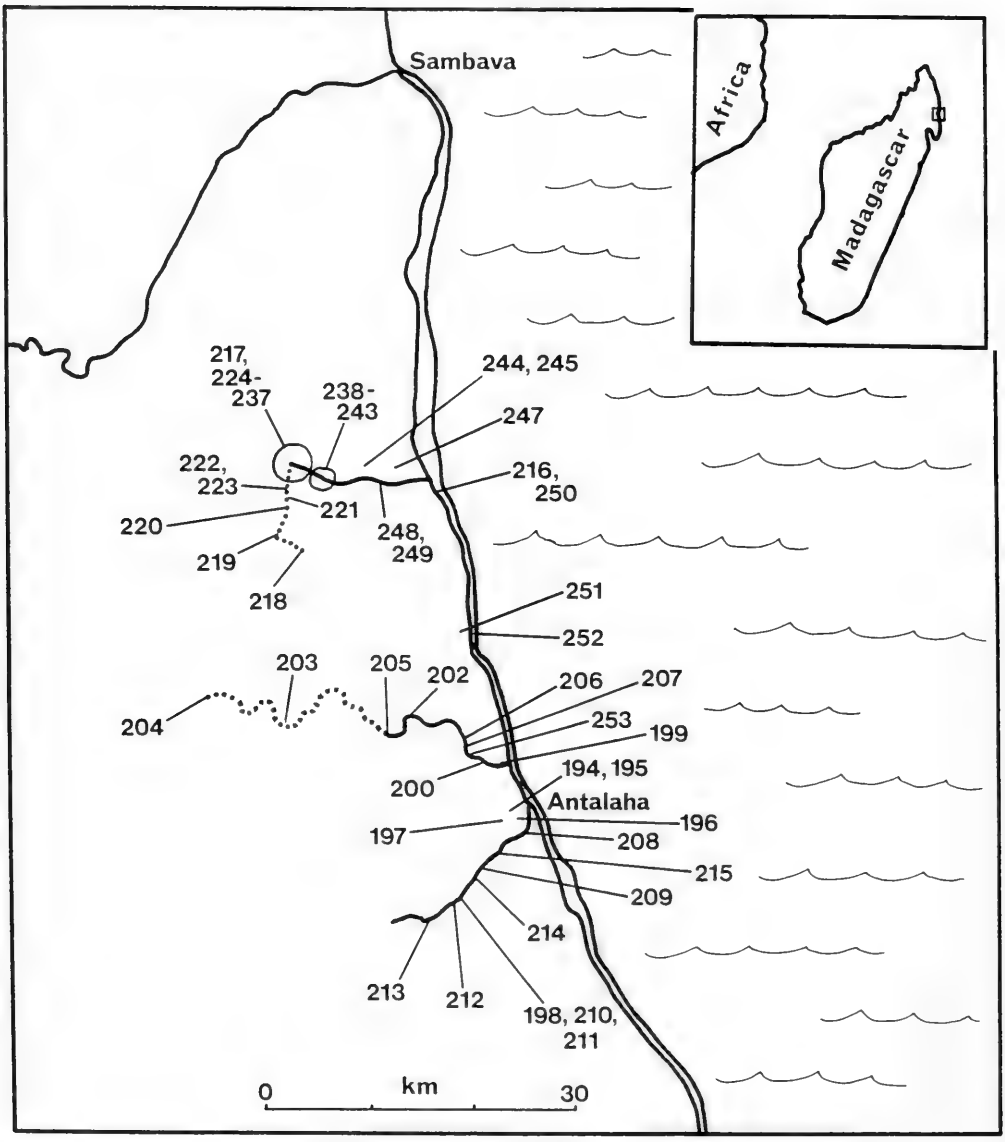

FIG. 1. Map of stations. Adapted from Emberton (1994).

ing the dry season to aid travel on the unpaved roads (Bradt, 1992). Transportation was by four-wheel-drive vehicle as far as possible (Fig. 1, solid lines), then on foot (dotted lines) for the two northern transects. Whenever a village was encountered, residents were informed via translator of the author's intent to purchase land snails collected from native forests, with bonuses for live specimens; a shell of the introduced and common *Achatina* was shown as the single kind not wanted. Purchases were made along the return trip, questioning the collectors for precise locality information (type of forest and its direction and distance from the village, plus date and time of collection). Additional collections were made away from villages with the aid of two assistants and whoever else could be recruited from the area. Land clearing, which eradicates native land

snails (Emberton, in prep.), was extensive along all three transects and limited the numbers and distributions of collection stations; some villages made no collections because of local festivals or because forests were considered too inaccessible.

The 39 stations listed in Appendix I produced acavid snails. Station numbers (in the KCE series) provide access to the computer-cataloged vouchers at the Academy of Natural Sciences of Philadelphia. Station coordinates and elevations were estimated from topographic maps.

Collections were sorted to acavid species and counted for live and dead specimens.

Stations were ranked for each of the three variables latitude, inland distance, and elevation. Latitude distinguished among the three transects: (1) northern = Andempona River valley and environs, (2) middle = Ankavanana River valley plus coastal station 252, and (3) southern = Ankavia River valley and environs. Inland distance categorized minimum straight-line distance to the coast into (1) 0–2.9 km, (2) 3–11.9 km, and (3) 12+ km. Elevation was also assigned three ranks: (1) 0–19.9 m, (2) 20–49.9 m, and (3) 50–80 m.

Species distributions were assessed by analyses of variance (ANOVAs) (Sokal & Rohlf, 1969). Station species counts (live plus dead) were transformed to proportions of total station acavids. These proportions were used as dependent variables for (1) one-way ANOVAs with treatment = elevation, (2) two-way ANOVAs with treatments = latitude and inland distance, and (3) two-way ANOVAs with treatments = latitude and elevation. Three-way ANOVAs and two-way ANOVAs with treatments = inland distance and elevation were not possible because of empty treatment cells. ANOVAs were by least-squares linear likelihood estimates, using SYSTAT multivariate general linear hypothesis programs (Wilkinson, 1988). Computer outputs of partitioned sums of squares were used to calculate the proportions of total species-distribution variance explained by treatments and their interaction, and unexplained, using a hand calculator. ANOVA cell means for each species were computed using SYSTAT (Wilkinson, 1988).

Distribution maps for the seven acavid species were prepared by combining present data with localities listed in Fischer-Piette et al. (1973). Antalaha-region distributions were compared with total Madagascan distributions as summarized by Emberton (1990).

RESULTS

Figure 2 illustrates (in a phylogenetic context) the seven acavid species that were found, and Table 1 lists the number of each species collected at each station. A total of 4,446 specimens was collected, with individual station collections ranging from two to 2,297. Maximum acavid site-diversity was four species (stations 206, 245–249). (Non-acavid large land snails of the helicarionid genus *Kalidos* and the prosobranch genera *Acroptychia* and *Tropidophora* were also collected in abundance; several stations were also collected for small to minute snails [Emberton, 1994].)

Ranks of each station for latitude, inland distance, and elevation are given in Table 1. One-way ANOVAs for elevation showed significant treatment effects in only one species, *Clavator moreleti,* for which elevation explained 31% of distributional variance ($p < 0.001$). *Clavator moreleti* was more abundant proportionally at lower elevations, with ANOVA cell means:

Elev. Rank	Cell Count	Mean Proportion
1	10	0.36
2	21	0.01
3	8	0.08

Two-way ANOVAs with treatments = latitude and inland distance showed significant treatment effects for five of the seven acavid species (Table 2). Table 3 gives cell sizes and cell mean proportions for each species from these ANOVAs. *Ampelita julii* was significantly stratified by latitude (Table 2), representing a fourth of all collections along the entire southern transect, but absent from all but some inland stations of the northern and middle transects, where it averaged less than a tenth of collections (Table 3). *Ampelita julii*'s presumed sister species, *A. soulaiana,* was significantly (Table 2) restricted to the stretch between 3 and 11.9 km inland along the Andempona River valley (= northern transect) (Table 3). Combining these two species as *A. (Eurystyla)* removed significant inland-distance × latitude interaction effects but not significant latitudinal effects, which explained 26% of the distribution of this subgenus (Table 2).

Ampelita xystera was significantly affected by inland distance, averaging a fourth or

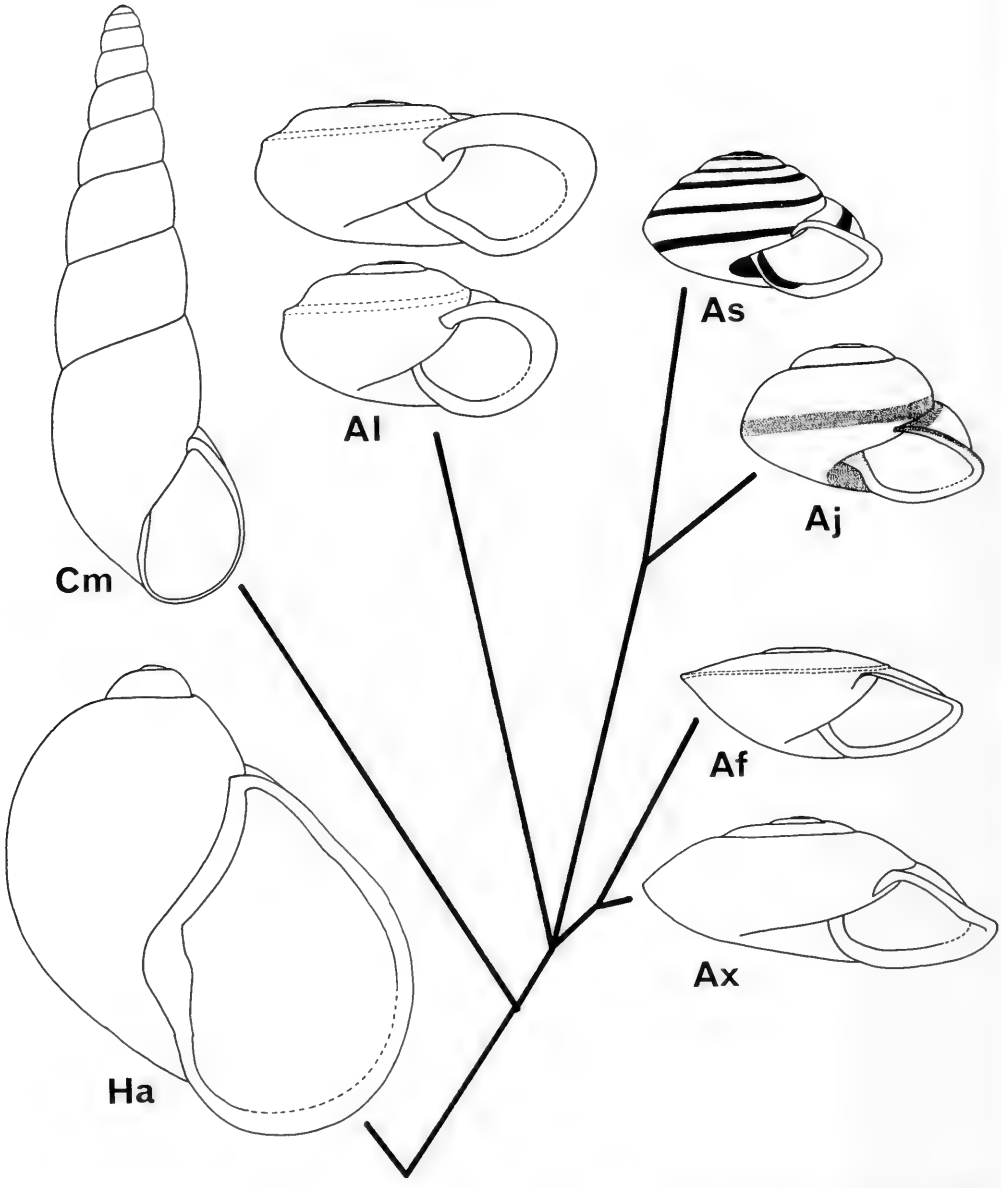

FIG. 2. Camera lucida drawings of seven species of acavid land snails from the region of Antalaha, northeastern Madagascar (Fig. 1). All are to the same size scale; Cm is 70.0 mm in height. Al = *Ampelita (Ampelita) lamarei* (two shells showing variation, station 205, Academy of Natural Sciences of Philadelphia [ANSP] catalog number 391391), Aj = *A. (Eurystyla) julii* (206, ANSP 391399), As = *A. (E.) soulaiana* (248, ANSP 391411), Ax = *A. (Xystera) xystera* (206, ANSP 391429), Af = *A. (X.) fulgurata* (205, ANSP 391434), Cm = *Clavator moreleti* (206, ANSP 391452), Ha = *Helicophanta amphibulima* (204, ANSP 391482). The phylogeny is based on allozymes for Ha, Cm, Al, Aj, and Ax (Emberton, in review b); and on shell similarity for As and Af (Emberton, 1990).

more of collections 3–12 km from the coast, but virtually absent from both coastal and more inland stations; latitudinal effects were not statistically significant, despite the fact that no *A. xystera* were collected from the southern transect. The related *A. fulgurata*

TABLE 1. Numbers of acavid snails collected at 39 stations in the region of Antalaha, northeastern Madagascar

				Ampelita										Clavator		Helicophanta		
				lamarei		julii		soulaiana		xystera		fulgurata		moreleti		amphibulima		
Sta #	Lat	Inl	Elv	L	D	L	D	L	D	L	D	L	D	L	D	L	D	Total
216	1	1	2	0	29	0	0	0	0	0	0	0	0	0	13	0	3	45
250	1	1	3	0	0	0	0	0	0	0	0	0	0	11	26	0	22	59
244	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3
245	1	2	2	0	8	0	0	0	5	0	28	0	0	0	0	0	2	43
248	1	2	2	0	7	0	0	0	5	0	8	0	0	0	0	0	5	25
249	1	2	2	0	4	0	0	0	6	0	9	0	0	0	0	1	72	92
246	1	2	3	0	4	0	0	0	7	0	12	0	0	0	0	0	6	29
247	1	2	3	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
223	1	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	21	21
225	1	3	2	0	4	0	1	0	0	0	0	0	0	0	0	0	0	5
226	1	3	2	0	10	0	0	0	0	0	0	0	0	0	0	0	0	10
229	1	3	2	0	2	0	0	0	0	0	0	0	0	0	0	0	4	6
232	1	3	2	0	1	0	1	0	0	0	0	0	0	0	0	0	8	10
233	1	3	2	0	2	0	2	0	0	0	0	0	0	0	0	0	25	29
234	1	3	2	0	12	0	6	0	0	0	0	0	0	0	0	1	97	116
235	1	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	8	9
236	1	3	2	0	7	0	2	0	0	0	0	0	0	0	0	0	1	10
237	1	3	2	1	16	0	5	0	0	0	0	0	0	0	0	0	43	65
238	1	3	2	0	16	0	0	0	0	0	0	0	0	0	0	0	1	17
239	1	3	2	0	6	0	0	0	0	0	12	0	0	0	0	0	0	18
240	1	3	2	0	89	0	0	0	0	0	0	0	0	0	0	0	41	130
241	1	3	2	0	41	0	0	0	0	0	0	0	0	0	0	0	0	41
243	1	3	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	2
218	1	3	3	0	6	0	0	0	0	0	0	0	0	0	0	0	1	7
224	1	3	3	0	8	0	0	0	0	0	0	0	0	0	0	0	100	108
200	2	1	1	0	0	0	0	0	0	0	0	0	0	7	2	0	0	9
252	2	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
201	2	2	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	3
206	2	2	1	0	224	0	202	0	0	42	614	0	0	97	1118	0	0	2297
207	2	2	1	0	0	0	2	0	0	0	60	0	0	17	330	0	0	409
253	2	2	1	0	0	0	0	0	0	0	46	0	0	40	23	0	0	109
204	2	3	3	6	60	0	0	0	0	0	0	0	0	0	0	1	299	366
205	2	3	3	3	92	2	53	0	0	0	0	0	65	0	1	0	50	266
208	3	1	3	0	5	0	2	0	0	0	0	0	0	0	0	0	0	7
210	3	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	2
211	3	2	1	0	1	0	14	0	0	0	0	0	0	0	0	0	21	36
214	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	8	8
215	3	2	2	0	2	0	4	0	0	0	0	0	0	0	0	0	1	7
212	3	3	1	0	7	0	6	0	0	0	0	0	0	0	0	0	10	23
Total				12	669	2	300	0	23	42	790	0	65	173	1514	3	853	4446
Live/Dead				0.02		0.01		0.00		0.05		0.00		0.11		0.00		

Stations are numbered as in Fig. 1 and are arranged geographically by latitude (Lat: 1, 2, and 3 = north, middle, and south transects), inland distance (Inl: 1, 2, and 3 = 0–2.9, 3–11.9, and 12+ km from coast), and elevation (Elv: 1, 2, and 3 = 0–19.9, 20–49.9, and 50–80 m). D = dead, L = live.

was collected only at station 205 (middle transect, inland distance 12+ km), where it comprised 12% of the collection. Combining these two species as *A. (Xystera)* eliminated significant spatial heterogeneity (Table 2).

Clavator moreleti was significantly affected by both latitude and inland distance as well as by their interaction. Although it was absent or virtually absent from the entire southern transect, from the far-inland middle transect, and from the mid- and far-inland northern transect, it averaged half or more of total collections from other regional divisions (Table 3). Thus *C. moreleti* in this study was restricted to and dominant at the mouth of the Andempona River valley (< 3 km from the

TABLE 2. Variances in species' proportions explained by latitude, by distance from the coast, and by their interaction

	Latitude	Inland	Lat*Inl	Unexpl.
A. lamarei	0.01	0.09	0.04	0.85
A. julii	0.35***	0.02	0.01	0.63
A. soulaiana	0.08	0.08	0.23*	0.61
A. xystera	0.03	0.16*	0.07	0.74
A. fulgurata	0.13*	0.11*	0.29**	0.48
C. moreleti	0.23***	0.15**	0.30***	0.32
H. amphibulima	0.03	0.07	0.07	0.82
A. (Eurystyla)	0.26**	0.01	0.05	0.68
A. (Xystera)	0.05	0.13	0.06	0.76

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (ANOVAs, df 2, 30 or [interactions] 4, 30).

TABLE 3. Cell means from the ANOVAs of Table 2

Cell Sizes				
		Inland		
		1	2	3
	1	2	6	17
Lat	2	2	4	2
	3	1	4	1
Ampelita (A.) lamarei				
	1	0.32	0.28	0.49
Lat	2	0.50	0.02	0.27
	3	0.71	0.20	0.30
A. (Eurystyla) julii				
	1	0.00	0.00	0.04
Lat	2	0.00	0.02	0.10
	3	0.29	0.24	0.26
A. (E.) soulaiana				
	1	0.00	0.10	0.00
Lat	2	0.00	0.00	0.00
	3	0.00	0.00	0.00
A. (Xystera) xystera				
	1	0.00	0.25	0.04
Lat	2	0.00	0.30	0.00
	3	0.00	0.00	0.00
A. (X.) fulgurata				
	1	0.00	0.00	0.00
Lat	2	0.00	0.00	0.12
	3	0.00	0.00	0.00
Clavator moreleti				
	1	0.46	0.00	0.00
Lat	2	0.50	0.66	0.00
	3	0.00	0.00	0.00
Helicophanta amphibulima				
	1	0.22	0.37	0.43
Lat	2	0.00	0.00	0.50
	3	0.00	0.56	0.44

coast) along the lower Ankavanana River valley (< 12 km from the coast).

Neither *Ampelita lamarei* nor *Helicophanta amphibulima* showed significant effects of latitude or inland distance (Table 2). *Ampelita lamarei* was extremely widespread, with no tendencies toward geographical trends. *Helicophanta amphibulima,* on the other hand, was conspicuously (though not significantly in a statistical sense) more common at greater distances inland. This trend was most pronounced in the middle transect, where *H. amphibulima* was found only at the most inland station; was distinct in the southern transect, where it did not appear in the most coastal station; and was subtle in the northern transect, where it graded from 22% to 37% to 43% of collections going inland.

Two-way ANOVAs with treatments = inland distance and elevation had the following cell counts:

		Inland Distance		
		1	2	3
	1	2	1	2
Elevation	2	7	5	2
	3	1	15	4

In these ANOVAs, no species showed significant treatment effects from elevation or from inland × elevation interaction. Only two species showed significant effects from inland distance: *Ampelita soulaiana* ($p < 0.01$, 24% of variance explained) and *Clavator moreleti* ($p < 0.05$, 17% of variance explained). When *A. soulaiana* was combined with *A. julii* in *A. (Eurystyla),* no significant treatment effects remained. High but less than significant inland-distance effects were evident for *A. xystera* ($0.05 < p < 0.10$, 16% of variance explained).

Figure 3 combines locality data from this study with those of Fischer-Piette et al. (1973) to produce distributional maps for the seven species. Adding the 20-year-old localities generally supports the results of this analysis. Thus *Ampelita lamarei* and *Helicophanta amphibulima* are both widespread and unlocalized in the region, although the former was found at more of the distant inland sites. The northern coastal sites for *H. amphibulima* (Fig. 3) were on hills (Fischer-Piette et al., 1973; Table 1); no elevational data were given for this species's two southern coastal sites.

Clavator moreleti in the region of Antalaha is clearly a species of the coast and the lower river valleys (Fig. 3). Fischer-Piette et al.'s (1973) locality of "Antsiranamatso" is inter-

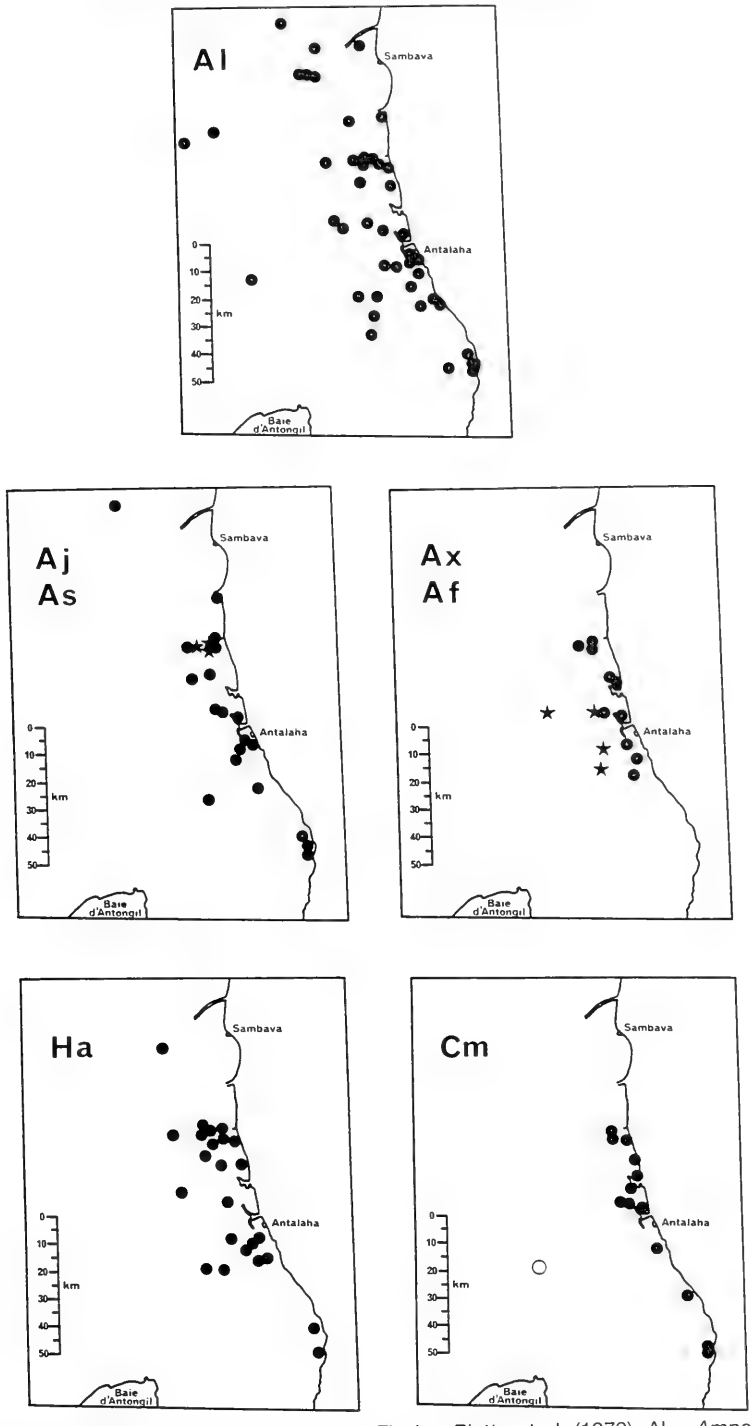

FIG. 3. Distribution maps based on this study and Fischer-Piette et al. (1973). Al = *Ampelita (Ampelita) lamarei,* Aj = *A. (Eurystyla) julii* (dots), As = *A. (E.) soulaiana* (stars), Ax = *A. (Xystera) xystera* (dots), Af = *A. (X.) fulgurata* (stars), Cm = *Clavator moreleti* (open circle = dubious record), Ha = *Helicophanta amphibulima.*

preted here as spurious (Fig. 3, open circle).

Ampelita julii's significant trend of occurring farther inland south of Antalaha (Table 3) is supported by the additional southwestern site of Antsambalahy, but is strongly negated by the extreme northwestern site of Amboahangibe (Fig. 3; Fischer-Piette et al., 1973). The seemingly related *A. soulaiana* is known from only three localites along a short stretch of the Andempona River valley, where it is closely bounded both upstream and downstream by *A. julii* (Fig. 3).

Ampelita xystera's inland distributional limit of approximately 12 km is well supported by the seven additional localities provided by Fischer-Piette et al. (1973) (Fig. 3). The coastal limit of 3 km (Table 3) is negated, however, by one of these localities: Antseranambidy (1 km south of Ampahana), a village within 0.2 km of the coast. *Ampelita xystera*'s probable relative, *A. fulgurata,* is known from only five localities, four of which are mapped in Figure 3. The fifth locality, "Ambohitsitandrona, 700 m" (Fischer-Piette, 1952), lies somewhere on the Masoala Peninsula, either south of Antalaha as mapped by Fischer-Piette (1952: fig. 1, #6), or on the peninsula's west coast (along the Baie d'Antongil, Fig. 3) near Mahalevona or near Ambanizana (Viette, 1991). There is a distinct geographical segregation between *A. fulgurata* and *A. xystera: fulgurata* occurs more inland and upland, but comes very close to *xystera* in the Ankavanana River valley (Fig. 3).

DISCUSSION

The area around Antalaha is now Madagascar's most intensively surveyed region for acavid land snails. This study, by hiring native collectors as much as possible, includes the largest collections of acavids ever made in Madagascar. Acavoids are among the world's largest, most ancient, relict, and K-selected non-orthurethran stylommatophoran snails, and Madagascar contains the greatest surviving radiation of acavoids (Emberton, 1990, in review a). Madagascar's environmental crisis (Myers, 1988; Green & Sussman, 1990) may put some of its acavids in danger of extinction, despite its system of Reserves and National Parks (Nicoll & Langrand, 1989).

Helicophanta amphibulima seems safe from extinction at present. Although Emberton (1990) mapped its distribution along Madagascar's entire western length (following Fischer-Piette, 1950), this species is also known in the east from Analamazoatra (= Perinet Reserve; Fischer-Piette & Garreau de Loubresse, 1965) and from the Antalaha area (Fischer-Piette et al., 1973; this study). Thus *H. amphibulima* is widespread and is protected in several reserves. In addition, as discovered in this study, it has a broad ecological tolerance and a widespread local distribution.

Clavator moreleti is probably endangered in the region of Antalaha, because of current deforestation of its coastal and river-mouth habitats. In the north, however, *C. moreleti* has been collected from high elevations that are under protection (Montagne d'Ambre, Mont Tsaratanana), as well as on Nosy Be, where it may be protected in Lokobe Reserve (Fischer-Piette & Salvat, 1963). The coastal distribution of this species in the Antalaha region is enigmatic, and could be due to its colonization history: perhaps *C. moreleti* invaded the region relatively recently along the coast, so has not had enough time to penetrate far inland.

Ampelita (A.) lamarei is widely distributed in northern and eastern Madagascar (Fischer-Piette, 1952; Emberton, 1990), and its broad local distribution (this study) further protects it from extinction.

Ampelita (Eurystyla) julii could be endangered. Its type—and sole western—locality, Ambanja (opposite Nosy Be; Fischer-Piette, 1952), is probably deforested by now, and none of its eastern localities (Fig. 3, plus Maroantsetra, at the head of Baie d'Antongil) falls under protection and will be deforested within a decade or two. It can be reasonably hoped, however, that *A. julii* occurs within Marojezy Reserve (northwest of Antalaha) or within what will hopefully become Masoala National Park (southwest of Antalaha). The significantly increasing abundance of *A. julii* toward the south in this study suggests that its range does extend more to the south, and that it has only relatively recently spread (and speciated) northward.

Ampelita (E.) soulaiana is definitely endangered. This taxon apparently represents a recent speciation or subspeciation event within the range of *A. julii,* but no live-collected specimens are available to test this hypothesis. The extremely small range of *A. soulaiana* (Fig. 3) will almost certainly be deforested in the near future.

Ampelita (Xystera) xystera is safe as a spe-

cies, as it has the widest known range of any Madagascan acavid (most of the northern half plus all the eastern rainforest). Its 12-km inland limit in the Antalaha region may mean its eventual local eradication; this limit may be due to climatic exclusion, "overcome" by speciation to form the inland *A. (X.) fulgurata* (see below). *Ampelita xystera*'s current 3-km coastal limit in the Antalaha area suggests advancing eradication by coastal deforestation. Antalaha *A. xystera* are well distinguished genetically from other regions, and are more plesiomorphic phylogenetically (Emberton, in review b), so should be saved.

Ampelita (X.) fulgurata is probably endangered. All of its four exactly known localities are unprotected and certain to be deforested within the next few years, despite its inland range (Fig. 3). The inland parapatry of this range relative to the related *A. xystera* suggests a parapatric speciation event as the genesis of *A. fulgurata;* other species with similar shells are widely separated geographically (Fischer-Piette, 1952; Emberton, 1990). The range given for *A. fulgurata* in Emberton (1990) was too broadly interpreted. *Ampelita fulgurata*'s fifth locality of "Ambohitsitandrona" was interpreted by Fischer-Piette (1952) as an unprotected site south of Antalaha, but there are two mountains so named on the western Masoala Peninsula, providing hope that *A. fulgurata* has a wide enough range to fall under the protection of a proposed Masoala National Park. The only locality where *A. fulgurata* has been collected in the past 20 years is station 205 (Fig. 1); this species has never been collected alive.

In sum, the distributional patterns of Antalaha-area acavid snails are significantly different and can be explained by historical founding events (*Clavator moreleti* and *Ampelita julii*), by climatic exclusion and coastal land clearing (*Ampelita xystera*), and by local speciation events (*A. soulaiana* and possibly *A. fulgurata*). This area is unprotected and is undergoing rapid deforestation, definitely endangering *A. soulaiana* and possibly endangering *A. julii, A. fulgurata,* and the genetically distinct and plesiomorphic local race of *A. xystera.*

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Revised Ms accepted 9 February 1994

APPENDIX I

200. 14.52.05S 50.14.40E, elev. approx. 10 m, approx. 1 km W of Andripika (approx. 6 km NW of Antalaha), remnant degraded forest on partially cleared and planted hillside beside road and river, 13 Oct. 1990, 3.0 person-hours.

201. 14.51.40S 50.13.40E, elev. approx. 10 m, approx. 2 km E of Valambana (approx. 7 km NW of Antalaha), talus and boulders beside road and river, 13 Oct. 1990, 1.5 person-hours.

204. 14.48.20S 49.59.10E, elev. approx. 50 m, northwest spur of Sarahandrano Peak, within bend of Ankavanana River, near Andranofotsy (WNW of Antalaha), virgin forest on slopes and ridgetop, 14 Oct. 1990, >10 person-hours.

205. 14.50.00S 50.08.25E, elev. approx. 50 m, region of Antsahanoro (approx. 17 km WNW of Antalaha), native forest, 13–15 Oct. 1990, >20 person-hours.

206. 14.50.20S 50.12.45E, elev. approx. 10 m, vicinity of Malotrandrohely (approx. 17 km WNW of Antalaha), native forest, 13–15 Oct. 1990, >20 person-hours.

207. 14.50.50S 50.13.00E, elev. approx. 10 m, "Bimanary, 40 m W of Valambanina" (approx. 10 km NW of Antalaha), native forest, 16 Oct. 1990, >10 person-hours.

208. 14.55.25S 50.16.25E, elev. approx. 50 m, region of Andrakarakani-ali, approx. 2 km SSW of Antalaha, 12–17 Oct. 1990, >20 person-hours.

210. 14.59.15S 50.12.50E, elev. approx. 10 m, region of Antserasera (approx. 12 km SW of Antalaha), 12–17 Oct. 1990, >10 person-hours.

211. 14.59.15S 50.12.50E, elev. approx. 10 m, region of Antserasera (approx. 12 km sw of Antalaha), 12–17 Oct. 1990, >10 person-hours.

212. 14.59.15S 50.12.00E, elev. approx. 10 m, region of Ambodimanga (approx. 15 km SW of Antalaha), 12–17 Oct. 1990, >10 person-hours.

214. 14.58.20S 50.13.25E, elev. approx. 10 m, "Mahatsara" (Mahasoa?), near Antserasera (approx. 10 km SW of Antalaha), 17 Oct. 1990, approx. 3 person-hours.

215. 14.56.25S 50.15.10E, elev. approx. 20 m, Andrakarakan' 1, approx. 3 km NE of Ambohitsara (approx. 8 km SW of Antalaha), approx. 0.5 km south of road, patch of virgin forest on hillside and hilltop, 17 Oct. 1990, 4 person-hours.

216. 14.37.35S 50.11.10E, elev. approx. 20 m, Ambohimanodina (hill), approx. 1.2 km S of Ambodipont-Sahana (approx. 35 km N of Antalaha), burn on edge of impacted forest, 18 Oct. 1990, 0.5 person-hours.

218. 14.40.00S 50.03.50E, elev. approx. 80 m, approx. 2–3 km E of Andampibe (7 km S of Lanjarivo, and approx. 40 km NNW of Antalaha), virgin dry pandanus-palm forest on quartz sand, 18 Oct. 1990, approx. 8 person-hours.

223. 14.36.55S 50.03.25E, elev. approx. 30 m, vicinity of Mangatsahatsa (1.5 km S of Lanjarivo, and approx. 40 km NNW of Antalaha), native forest, 19 Oct. 1990, aprox. 6 person-hours.

224. 14.36.15S 50.02.50E, elev. approx. 50 m, 2 km NW of Mangatsahatsa (1.5 km S of Lanjarivo, and approx. 40 km NNW of Antalaha): native forest, 18 Oct. 1990, approx. 6 person-hours.

225. 14.35.05S 50.03.20E (Lanjarivo), elev. approx. 35 m, approx. 4 km NW of Lanjarivo (approx. 40 km NNW of Antalaha), native forest, 18 Oct. 1990, approx. 9 person-hours.

226. 14.36.05S 50.03.20E (Lanjarivo), elev. approx. 35 m, approx. 2 km ENE of Lanjarivo (approx. 40 km nnw of Antalaha), virgin forest, 19 Oct. 1990, approx. 4 person-hours.

229. 14.36.05S 50.03.20E (Lanjarivo), elev. approx. 35 m, approx. 2 km N of Lan-

jarivo (approx. 40 km NNW of Antalaha), partially cleared forest, 19 Oct. 1990, approx 4 person-hours.

232. 14.36.05S 50.03.20E (Lanjarivo), elev. approx. 35 m, approx. 3 km w of Lanjarivo (approx. 40 km NNW of Antalaha), partially cleared forest in different place from 233, 19 Oct. 1990, approx. 2 person-hours.

233. 14.36.05S 50.03.20E (Lanjarivo), elev. approx. 35 m, approx. 3 km W of Lanjarivo (approx. 40 km NNW of Antalaha), partially cleared forest in different place from 232, 19 Oct. 1990, approx. 4 person-hours.

234. 14.36.05S 50.03.20E (Lanjarivo), elev. approx. 35 m, approx. 3 km w of Lanajarivo (approx. 40 km nnw of Antalaha), virgin forest, 19 Oct. 1990, approx. 10 person-hours.

235. 14.36.05S 50.03.20E (Lanjarivo), elev. approx. 35 m, approx. 3 km sw of Lanjarivo (approx. 40 km nnw of Antalaha), virgin forest, 18 Oct. 1990, approx. 3 person-hours.

236. 14.36.05S 50.03.20E (Lanjarivo), elev. approx. 35 m, approx. 2 km NW of Lanjarivo (approx. 40 km nnw of Antalaha), virgin forest, 18 Oct. 1990, approx. 2 person-hours.

237. 14.36.05S 50.03.20E (Lanjarivo), elev. approx. 35 m, region of Lanjarivo (approx. 40 km NNW of Antalaha), forest, 18–19 Oct. 1990, >20 person-hours.

238. 14.36.30S 50.04.40E (Ambodilalona), elev. approx. 30 m, between "Ankorakabe" and Ambodilalona (approx. 40 km NNW of Antalaha), along path: virgin forest, 19 Oct. 1990, approx. 5 person-hours.

239. 14.36.30S 50.04.40E (Ambodilalona), elev. approx. 30 m, approx. 4 km N of Ambodilalona (approx. 40 km nnw of Antalaha), virgin forest, 19 Oct. 1990, approx. 2 person-hours.

240. 14.36.30S 50.04.40E (Ambodilalona), elev. approx. 30 m, region of Ambodilalona (approx. 40 km NNW of Antalaha), forest, 18–19 Oct. 1990, >20 person-hours.

241. 14.36.30S 50.04.40E (Ambodilalona), elev. approx. 30 m, approx. 2 km N of Ambodilalona (approx. 40 km NNW of Antalaha), virgin forest, 19 Oct. 1990, approx. 4 person-hours.

243. 14.36.30S 50.04.40E (Ambodilalona), elev. approx. 30 m, approx. 2 km W of "Ambosimila" (unmapped village approx. 1 km E of Ambodilalona, approx. 40 km nnw of Antalaha), virgin forest, 19 Oct. 1990, approx. 1 person-hour.

244. 14.36.40S 50.07.15E (Ambinanifaho), elev. approx. 30 m, approx. 4 km SW of Ambinanifaho (approx. 36 km NNW of Antalaha), virgin forest, 18 Oct. 1990, approx. 2 person-hours.

245. 14.36.40S 50.07.15E (Ambinanifaho), elev. approx. 30 m, region of Ambinanifaho (approx. 36 km NNW of Antalaha), virgin forest, 18 Oct. 1990, >20 person-hours.

246. 14.38.00S 50.07.25E, elev. approx. 70 m, approx. 3 km S of Ambinanifaho (approx. 36 km NNW of Antalaha), partially cleared forest, 19 Oct. 1990, approx. 4 person-hours.

247. 14.36.30S 50.09.00E, elev. approx. 50 m, Beramboa (hill), approx. 2 km E of Ambinanifaho (approx. 36 km nnw of Antalaha), partially cleared forest and burn, 19 Oct. 1990, approx. 9 person-hours.

248. 14.37.20S 50.08.10E, elev. approx. 25 m, approx. 2 km E of Ambinanifaho (approx. 36 km NNW of Antalaha), south side of road: lowland virgin forest, 19 Oct. 1990, approx. 5 person-hours.

249. 14.37.20S 50.08.10E, elev. approx. 25 m, 7 km w of Ambodipont-sahana (approx. 35 km N of Antalaha), virgin forest, 19 Oct. 1990, approx. 4 person-hours.

250. 14.37.20S 50.11.05E, elev. approx. 50 m, Ambohimanodina (hill), 1 km S of Ambodipont-Sahana (approx. 35 km N of Antalaha), virgin forest near coast, 19 Oct. 1990, approx. 12 person-hours.

252. 14.44.10S 50.13.20E, elev. approx. 5 m, 1 km E of Tampolo, near coast (approx. 20 km N of Antalaha), on trees near shore. 18 Oct. 1990, approx. 8 person-hours.

253. 14.51.25S 50.13.00E, elev. approx. 10 m, 0.5 km W of Valambana (approx. 13.5 km NW of Antalaha), virgin forest, 18 Oct. 1990, approx. 6 person-hours.

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EFFECT OF TEMPERATURE ON REPRODUCTION IN *PLANORBARIUS CORNEUS* (L.) AND *PLANORBIS PLANORBIS* (L.) THROUGHOUT THE LIFE SPAN

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ABSTRACT

The reproduction of two planorbid species, *Planorbarius corneus* and *Planorbis planorbis,* was studied at 5, 10, 15, 20 and 25°C. All reproduction parameters were affected by temperature. *Planorbis planorbis* began to lay eggs from 10°C, whereas *P. corneus* reproduced from 15°C. Sexual maturity was earlier at higher temperatures. The snails spent most of their life span in reproduction (at least, 49% for *P. corneus* at 25°C, and 87% for *P. planorbis* at 15°C). Special attention was payed to abnormalities of eggs and egg capsules. *Planorbarius corneus* placed at 20°C and *P. planorbis* at 15°C produced the maximum number of descendants, 1,600 and 3,357 newly hatched snails per individual respectively.

Key words: Planorbidae, *Planorbarius corneus, Planorbis planorbis,* reproduction, temperature, ecophysiology.

INTRODUCTION

The reproduction of freshwater snails, and especially bilharziasis intermediate host snails, has been the subject of many papers (Cole, 1925; Chernin & Michelson, 1957; van der Schalie & Berry, 1973; Aboul-Ela & Beddiny, 1980; Seuge & Bluzat, 1983; Vianey-Liaud, 1990). However, these studies were limited in time and, to our knowledge, little quantitative data about the descendants of pluriannual species are available.

Planorbarius corneus and *Planorbis planorbis* are hermaphrodite freshwater snails. The complexity of their reproductive system is increased by internal fertilization, auto- and allosperm storage, egg capsule complexity, and autolysis of foreign sperm (Geraerts & Joosse, 1984). Eggs, which comprise a zygote surrounded by perivitelline fluid and membrane, are embedded in jelly and enclosed in a common egg capsule. Freshwater snails usually practise cross-fertilization (Duncan, 1975; Vianey-Liaud, 1990). *Planorbis planorbis* cannot be considered as a self-fertile species, whereas isolated *P. corneus* produces few capsules, eggs and egg cells (Costil, 1993).

Little is known about the ecophysiology of reproduction in these planorbid species, and the aim of this study is therefore to test the influence of temperature on:

age of snails at onset of sexual maturity;
reproduction-period duration;
number of eggs per capsule;
"abnormalities" of the capsules and the eggs;
variation of the numbers of capsules and eggs per individual; and
fecundity (number of eggs per individual) and fertility (number of newly hatched snails per adult) throughout the life span.

MATERIALS AND METHODS

Snails were collected in spring 1987 from two ponds located near Rennes, Brittany, France. Brought to the laboratory, they laid egg capsules. Reproduction was studied on snails hatched in the laboratory. The newly hatched snails were grouped in equal sized sets of 17 individuals (*P. corneus;* ANOVA: significance level of 95%: $N = 374$, $F = 1.84$, $p = 0.12$) or 20 specimens (*P. planorbis:* $N = 400$, $F = 0.01$; $p = 0.99$) at five constant temperatures: 5, 10, 15, 20 and 25°C. In the case of *P. corneus,* 374 individuals were reared as five sets at 5°C and 10°C, and four sets at 15, 20 and 25°C. For *P. planorbis,* 400 individuals were divided into four sets at each temperature.

At every temperature and according to mortality, the groups were adjusted to constant density. Between sets at different temperatures, plastic partitions were placed in the aquaria so that each snail could be in the same volume of water. Up to the age of ten months, they had a volume of 45 ml then 150 ml of pond water per individual, and they were fed with fresh lettuce *ad libitum* in a 12/12 h light/dark photoperiod.

Sexual maturity was considered acquired when the first egg mass was observed. Every

week, the survivors were counted, the egg capsules collected, and eggs counted under a binocular microscope. Each experiment continued until the death of the last snail. Abnormalities in the clutches were studied throughout the life of *P. corneus* reared at 20°C and 25°C, but only at fixed dates in the other cases.

At each temperature, the following parameters were computed:

- mean age (A_m) and, mean (D_m), minimum (D_{min}) and maximum (D_{max}) shell diameters at onset of sexual maturity;
- reproduction period duration, T_{rep} (in weeks);
- reproduction period duration in relation to maximum life span, T_{rep} (in %);
- mean numbers of egg capsules (N_c) and eggs (N_e) per snail alive per two weeks;
- mean number of eggs per egg capsule ($N_{e/c}$);
- fecundity of snails (Fec): cumulative number of eggs laid per snail throughout the life span; the fecundity per reproduction week is also calculated (Fec/T_{rep});
- proportion of capsules without eggs (in relation to N_c) (in %);
- proportion of capsules containing one or more eggs without egg cells (in relation to N_c) (C_w) (in %);
- proportion of eggs without egg cells (in relation to N_e) (E_w) (in %);
- proportion of eggs including two or more egg cells (in relation to N_e) (in %); and
- snail fertility (Fer): cumulative number of newly hatched snails produced per snail throughout the life span.

Considering the survivorship (Costil, 1994) and the reproduction of the planorbids, Leslie matrices were constructed (Leslie, 1945). The descending vertical elements represented the successive age classes (from birth to maximum four years). The probability of an individual surviving from age N to age N + 1 was stated on the diagonal, and mean individual fertility per year class on the top horizontal row.

RESULTS

Onset of Sexual Maturity

Planorbarius corneus laid egg capsules at temperatures of 15, 20 and 25°C, whereas *P. planorbis* reproduced from 10°C (Table 1). The higher the temperature, the faster the maturation was: 15th week at 25°C and 49th week at 15°C for *P. corneus.*

In *P. corneus,* the minimum, maximum and mean diameters were similar at 20°C and 25°C. At 15°C these values were higher and, on average, snails began their reproduction when they reached 13 mm. For *P. planorbis,* the mean diameter ranged from 5.4 to 6.9 mm.

Reproduction-Period Duration

In the laboratory, egg laying occurs throughout the year though this is not true for field populations. For both species considered together, the reproduction-period duration was negatively correlated with temperature (Kendall's correlation: $N = 7$, $\tau = -0.617$, $p = 0.05$), and positively correlated with maximum longevity ($\tau = 0.905$, $p = 0.04$). At 15°C, *P. corneus* reproduced for 175 weeks, corresponding to 75.8% of its maximum longevity, whereas *P. planorbis* laid eggs for 125 weeks, corresponding to 87.4% of its life.

Variation of the Number of Egg Capsules and Eggs

Temperature strongly influenced the reproduction of the two species, especially egg laying in *P. corneus.* At 20°C, a peak was observed from the 41st week to the 75th week, and the maximum production of eggs reached 8.5 capsules per snail per two weeks (Fig. 1). At 25°C, the snails produced a constant number of capsules (between 0.5 and 1/snail/2 weeks), and at 15°C, the maximum value was 3.2. At the latter temperature, an egg laying rhythm of 44 weeks was observed (periodogram method: Fourier's analysis, $p < 0.05$). Great variations of the number of eggs per capsule were noticed in *P. corneus.* At the two highest temperatures, the capsules had few eggs at the beginning of the reproduction period. The numbers of eggs per mass then increased to 21.5 (20°C, 131st week) or 17.8 (25°C, 57th week). The maximum number for snails reared at 15°C reached 15.4. There were significant Kendall's correlations between the number of laid capsules and the number of eggs per capsule at 20°C (positive correlation: $\tau = 0.425$, $p < 0.001$) and at 25°C (negative correlation: $\tau = -0.304$, $p < 0.03$).

In *P. planorbis,* capsule production varied from one week to the next irrespective of temperature, with maximum values of 6.8 (10°C), 7.4 (25°C), 8.6 (15°C) and 9.7 (20°C) (Fig. 2). Nevertheless, rhythms of 56 weeks (10°C) and 58 weeks (15°C) were found by

TABLE 1. Main features of the reproduction of *Planorbarius corneus* and *Planorbis planorbis* in relation to temperature (x ± standard deviation).

	Planorbarius corneus			*Planorbis planorbis*			
Temperature	15°C	20°C	25°C	10°C	15°C	20°C	25°C
Mean age at onset of sexual maturity (A_m) (weeks)	49	17	15	25	15	11	9
Mean diameter at onset of sexual maturity (D_m) ± s	12.8 ± 1.4	10.7 ± 0.8	10.5 ± 1.2	5.9 ± 0.8	6.9 ± 0.9	6.8 ± 0.6	5.4 ± 0.9
Minimum diameter (D_{min}) (mm)	9.9	7.6	7.8	2.9	4.6	5.7	3.3
Maximum diameter (D_{max}) (mm)	16.6	12.5	13.0	7.1	9.0	8.4	7.7
Reproduction period duration (T_{rep}) (weeks)	175	141	55	135	125	61	24
Maximum longevity (weeks)	231	203	113	175	143	83	39
Reproduction period duration in relation to maximum longevity, (T_{rep}) (weeks)	75.8	69.5	48.7	77.1	87.4	73.5	61.5
Number of egg capsules laid per snail throughout the life span	81	123	17	161	258	150	38
Number of egg capsules laid per snail per reproduction week	0.5	0.9	0.3	1.2	2.1	2.5	1.6
Number of eggs laid per snail throughout the life span (Fec)	644	1813	172	1160	3863	2855	329
Number of eggs laid per snail per reproduction week	3.7	12.9	3.1	8.6	30.9	46.8	13.7
Mean number of eggs per egg capsule (Ne/c) ± s	8.0 ± 4.9	14.8 ± 4.8	10.1 ± 7.1	7.2 ± 2.7	14.9 ± 3.8	18.9 ± 6.1	8.6 ± 5.0
Maximum number of eggs per egg capsule	27	41	48	20	36	46	31
Mean hatching rate (% ± s)	92 ± 11	94 ± 10	94 ± 8	0	89 ± 19	86 ± 18	90 ± 16

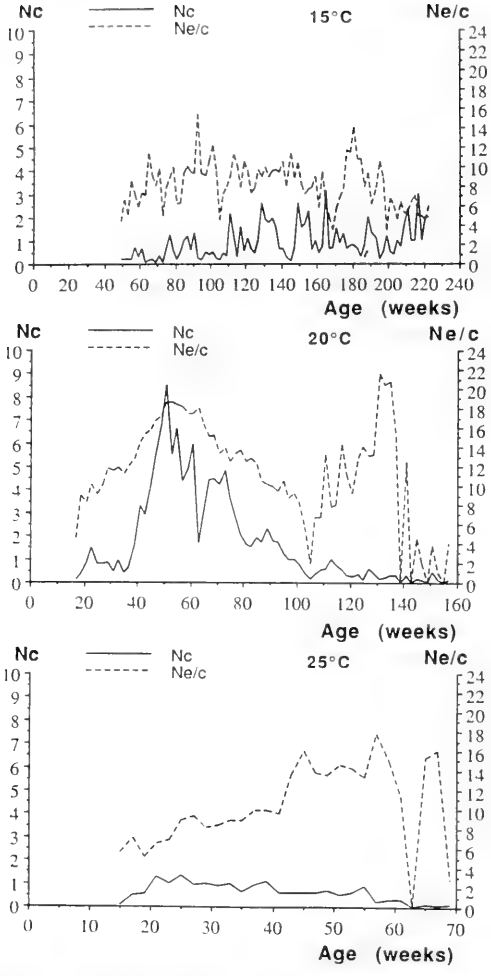

FIG. 1. Reproduction of *Planorbarius corneus* in relation to temperature and age: variation in the number of egg capsules per individual per 2 weeks (Nc); variation in the number of eggs per capsule (Ne/c).

periodogram analysis ($p < 0.05$). Between 15°C and 25°C, the number of eggs per capsule, low in the first weeks of the reproduction, increased for 12 to 18 weeks before stabilizing. At the end of the reproductive period, the number of the eggs per capsule of *P. planorbis* reared at 10°C cannot be well established because of small number of laid capsules. The number of eggs per mass produced by the snails at 15°C is positively correlated with the number of laid masses ($\tau = 0.348$, $p < 0.001$).

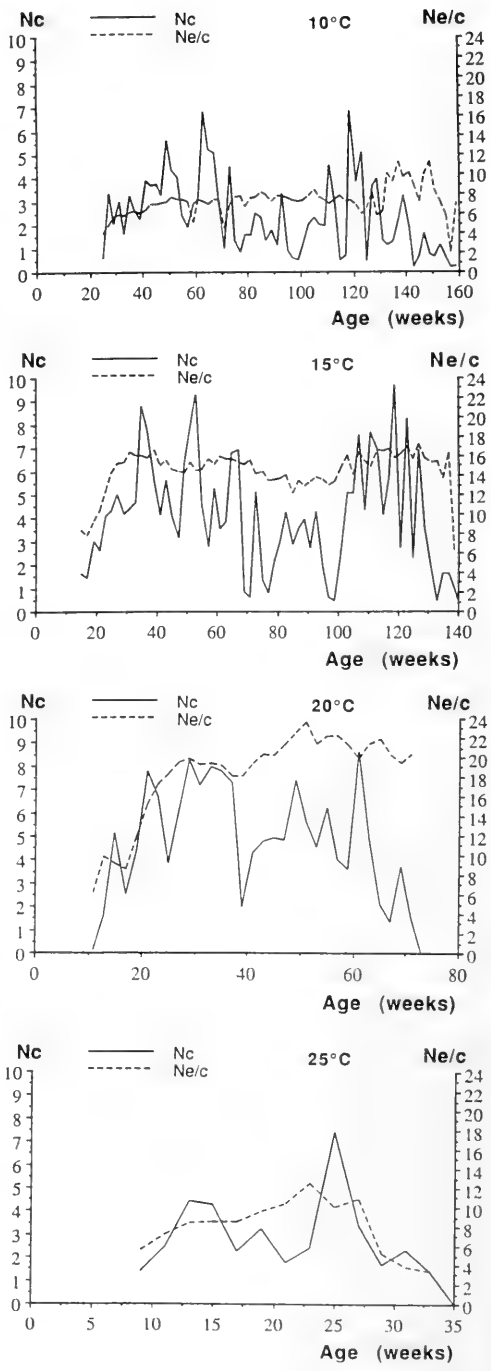

FIG. 2. Reproduction of *Planorbis planorbis* in relation to temperature and age: variation in the number of egg capsules per individual per 2 weeks (Nc); variation in the number of eggs per capsule (Ne/c).

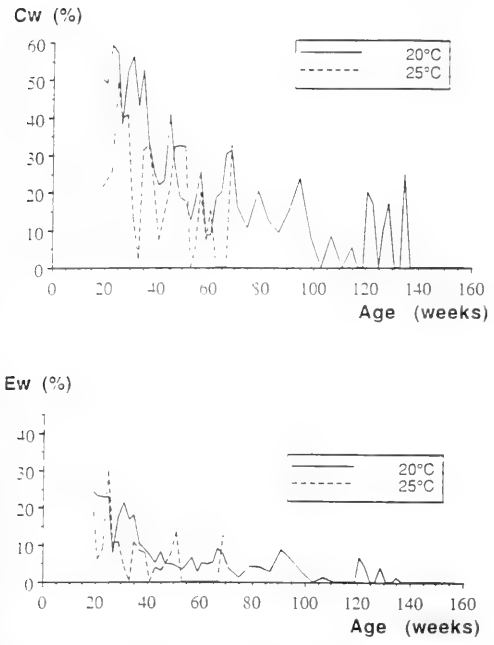

FIG. 3. Reproductive "abnormalities" of *Planorbarius corneus* reared at 20°C and 25°C: variation in the number of capsules including one or more eggs without egg cells (C_w); variation in the number of eggs without egg cells (E_w).

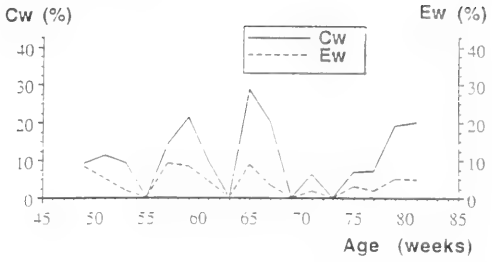

FIG. 4. Reproductive "abnormalities" of *Planorbarius corneus* reared at 15°C (from 49 to 81 weeks old): the number of capsules containing one or more eggs without egg cells (C_w); the number of eggs without egg cells (E_w).

"Abnormalities" of Egg Capsules and Eggs

Capsules were considered abnormal when they contained no egg, or when all or part of the eggs were without egg cells, i.e. zygotes. Moreover, the capsules with eggs containing more than one egg cell were also considered abnormal. Throughout the reproductive period of *P. corneus,* the numbers of capsules without eggs at 20°C and 25°C were respectively 125 (corresponding to 0.14%) and 17 (corresponding to 0.20%). The greatest percentages of capsules containing eggs without egg cells occurred at the beginning of reproduction (Fig. 3). The mean percentages of eggs without egg cells at 25°C and 20°C were respectively 7.1% (standard deviation, s = 7.5) and 5.5% (s = 6.8). For *P. corneus* reared at 15°C (age: 49–81 weeks), this value was 3.9% (s = 3.3) (Fig. 4), and 21 eggs with more than one egg cell occurred as twin eggs (16), three egg cells (3), four egg cells (1) and nine egg cells (1). At 20°C, the total number of eggs with multiple egg cells was twice what it was at 25°C (Table 2). Twins were particularly numerous but rarely developed further. Whatever the species, no eclosion occurred in eggs containing more than two egg cells.

Table 3 summarizes the results concerning *P. planorbis.* The percentage of capsules without eggs increased with time. Except at 10°C, the same phenomenon was observed for eggs without egg cells, which were especially numerous at 25°C. Two capsules without proper eggs but containing 16 and 18 egg cells were laid by snails reared at 10°C. A significantly greater number of eggs with several egg cells was laid at 10°C compared with higher temperatures ($H = 5.326$, $p = 0.15$; Kruskall & Wallis test). Nevertheless, even at 10°C, these eggs were not often noticed. Dwarf or giant eggs were encountered particularly at 10°C. The number of egg cells per egg never exceeded four, and twin egg cells were the most numerous.

Fecundity and Fertility

Planorbarius corneus was most fecund at 20°C (Table 1). The 6,031 capsules laid by all the snails contained on average 14.8 eggs, whereas this number was 8.0 for clutches produced at 15°C. In *P. planorbis,* the highest number of eggs per capsule was also observed at 20°C (18.9 ± 6.1). The temperature of 15°C may have allowed the snails to lay the maximum capsule number due to a lengthening of the life span and reproduction period. Nevertheless, fecundity related to reproduction week was maximum at 20°C (2.5 capsules and 46.8 eggs/individual/reproduction week). The temperatures of 25°C and 10°C appeared to be especially unfavorable for the reproduction of *P. planorbis.*

TABLE 2. "Reproductive abnormalities" in *Planorbarius corneus* reared at 20°C and 25°C: number of egg cells per egg.

	20°C		25°C	
Number of egg cells per egg	Number of eggs containing such a number of egg cells	Percentage of eggs containing such a number of egg cells	Number of eggs containing such a number of egg cells	Percentage of eggs containing such a number of egg cells
2	204	0.226	9	0.106
3	30	0.033	1	0.012
4	8	0.009	1	0.012
5	5	0.006	0	0
9	2	0.002	0	0
6, 10, 13 & 15	1	0.001	0	0
Total	253	0.280	11	0.130

The dynamics of pluriannual experimental populations was investigated using Leslie matrices (Table 4). At every temperature, the greatest reproductive effort was made by the penultimate age class. The total fertility of *P. planorbis* reared at 25°C was low (23 newly hatched snails per individual). The greatest fertility of *P. corneus* was at 20°C (1,600 individuals/snail), whereas *P. planorbis* produced the maximum number of newly hatched snails (3,357/adult) at 15°C. The eggs of *P. corneus* developed at 10°C, but this species did not reproduce at this temperature.

DISCUSSION

In contrast to *P. corneus, P. planorbis* developed but did not lay eggs at 10°C. However, for the embryonic development study, the eggs placed in incubation were laid at 20°C. It was possible that the temperature at which the eggs had been elaborated was important for the future development. Eggs of *P. planorbis* laid at 10°C could develop if they were then incubated at this temperature. So, the hatching rate could be slightly lower than the rate of *P. corneus* (35%), as it was the case for the other temperatures. The number of newly hatched snails could be 404.

The minimum threshold for the reproduction of temperate freshwater snails appears to be between 7°C and 12°C (Vaughn, 1953; Boerger, 1975; Duncan, 1975; Eversole, 1978; Krkac, 1982), and 12°C was also stated for *P. corneus* by Precht (1936). According to Joosse & Veld (1972), ovogenesis of *Lymnaea stagnalis* (L.) did not depend on temperature, and ovocytes of all stages were found in the ovotestis of infertile snails reared at 5°C or 8°C, although spermatogenesis stopped at these low temperatures. Eggs were produced by adults of *Lymnaea obrussa* Say at temperatures ranging from 10°C to about 26°C (Mattice, 1975). Krkac (1982) explained that egg laying of *Physa acuta* (Draparnaud) was stimulated by every temperature increase up to 30°C. At a temperature of 25°C, the maximum threshold for the reproduction of both studied species was not attained, and it could be slighly below 30°C. In the tropical species, *Biomphalaria glabrata* (Say), it reached 33°C (Vianey-Liaud, 1982).

The temperature effect on animal reproduction is both direct (existence of thresholds) and indirect (general development, growth). There is an acceleration of reproductive system maturation with temperature, a relationship not observed in some other freshwater pulmonates, including *Bulinus truncatus* (Audouin), *Biomphalaria alexandrina* (Ehrenberg) and *Helisoma duryi* (Wetherby) (El Eman & Madsen, 1982). Moreover, these snails laid their first egg masses when they attained a certain size but at very different ages (three weeks for the earlier *Bulinus* and five weeks for the earlier planorbids). In comparison with the two studied species, other species appear to reach sexual maturity earlier: *P. acuta,* five weeks (Perrin, 1986), *Lymnaea truncatula* (Müller), four weeks (Hodasi, 1976). Onset of sexual maturity depends on many factors. For example, in the laboratory, *Lymnaea peregra* (Müller) from exposed habitats initiated reproduction earlier and put more effort into it than snails from sheltered habitats (Calow, 1981).

Basommatophoran snails often reproduce

TABLE 3. Reproductive "abnormalities" of *Planorbis planorbis* in relation to age and temperature.

Temperature	Age (weeks)	Proportion of capsules without eggs (%)	Proportion of capsules including 1 or more eggs without egg cells (%)	Proportion of eggs without egg cells (%)	Proportion of eggs including 2 or more egg cells (%)
	29	0	20.5	8.1	0.43
	31	0	5.0	2.1	0.70
	33	0	4.6	2.1	0
	35	0	0	0	0.75
	37	0	0	0	0.30
	51	0	1.3	0.5	0.25
10°C	59	0.9	1.0	0.6	0
	77	3.4	3.6	0.5	2.30
	105	8.9	0	0	0.24
	113	17.2	0	0	0.26
	129	19.1	3.9	1.7	0
	141	20.7	0	0	0
	153	30.8	0	0	0
	31	0	0.7	0.3	0
	49	0	0.9	0.5	0
	57	0.5	0.5	0.2	0.04
	73	0.3	0.6	0.3	0
	85	0.6	1.1	0.6	0.05
	93	1.7	0.4	0.06	0.03
15°C	117	1.2	0.4	0.02	0.02
	123	1.5	1.2	0.3	0.04
	127	5.2	4.7	1.4	0
	131	33.3	20.0	3.9	0
	133	27.3	30.0	8.3	0
	135	33.3	33.0	8.5	0
	21	0.2	1.1	0.3	0.02
	35	0.4	0.4	0.1	0.02
	51	4.6	5.0	1.3	0
20°C	53	2.9	6.5	1.4	0
	59	8.5	11.1	2.9	0.04
	61	11.9	8.6	3.0	0
	63	11.1	19.3	5.6	0.11
	65	8.8	25.8	9.4	0
	15	0	11.2	4.5	0
	25	1.5	27	12.1	0.10
25°C	27	0	39.4	21.4	0
	29	0	90.6	65.2	0.60
	31	0	95.7	93.0	0
	33	0	100	100	0

before reaching half adult size, and growth then continues (Larambergue, 1939). Considering the maximum size when the planorbids produce their first egg capsules, we conclude that *P. planorbis* and *P. corneus* lay their first eggs at sizes of 7.1 mm and 12.5 mm respectively, and these sizes correspond to 0.5 or 0.4 times the observed maximum sizes.

As for many freshwater pulmonates, planorbid reproduction in experimental conditions is continuous throughout the year. In *P. planorbis* and *P. corneus,* the mean durations of the reproduction period were 74.9% and 64.7% of the maximum longevity respectively, compared with 63.6% in *H. duryi* (Aboul-Ela & Beddiny, 1980). For *P. planorbis,* the last capsule was laid shortly before death, whereas in *P. corneus* reared at 20°C and 25°C, reproduction stopped 40 weeks before. The latter snails might suffer from a gamete exhaustion and/or damage to the reproductive system.

Although few malacologists have reported

TABLE 4. Leslie matrices constructed for experimental populations of *Planorbarius corneus* and *Planorbis planorbis* reared at different temperatures. Fertility at the successive age classes (from birth up to 4 years) is on the horizontal axis; survivorship is on the diagonal. The numbers of the top vertical row represent the total fertility (including the new hatched snails produced at the end of the life, during the incomplete year). See "discussion" for the numbers in brackets stated for *P. planorbis* at 10°C.

		0	3	94	243	153	
		0.75	0	0	0	0	
	15°C	0	0.84	0	0	0	555
		0	0	0.63	0	0	
		0	0	0	0.11	0	
Planorbarius		0	636	890	74		
corneus	20°C	0.76	0	0	0		1600
		0	0.71	0	0		
		0	0	0.27	0		
		0	128	22			
	25°C	0.72	0	0			150
		0	0.06	0			
		0	0 (107)	0 (153)	0 (140)		
	10°C	0.44	0	0	0		0 (404)
Planorbis		0	0.63	0	0		
planorbis		0	0	0.21	0		
		0	1244	1059			
	15°C	0.84	0	0			3357
		0	0.69	0			
	20°C	0	1760				2389
		0.55	0				

abnormalities of eggs or egg masses (Bloch, 1938, for *P. corneus* and *L. stagnalis;* Bondesen, 1950, for *Ancylus fluviatilis* (Müller); Bigus, 1981, for *Physa acuta*), it is important to take such abnormalities into account if we do not want to overestimate the snail fertility. In our study, most of the eggs without an egg cell were laid at the beginning of the reproductive period, so the start-up of reproduction seemed to present some problems. However, the occurrence of capsules without eggs in *P. corneus* and *P. planorbis,* and of the eggs without egg cells in *P. planorbis,* increases with time. These eggs and capsules reflected a lack or a dysfunction in the formation of gametes and eggs. For individuals of *P. planorbis* of the same age, the reproduction problems were more numerous with higher temperatures. Vianey-Liaud (1982) showed that at 33°C the reversible sterilization of *B. glabrata* was not due to a problem of reproductive system differentiation, but to a disruption of the system functioning. By contrast, Michelson (1961) showed that in *B. glabrata* low temperatures reduced fecundity without obviously damaging the reproductive system, whereas high temperatures reduced the female sexual organs. The results reported here confirm the former rather than the latter author. In *Ancylus fluviatilis,* the percentage of abnormal capsules, eggs and egg cells varied from 10% to 24% according to year (Bondesen, 1950). Moreover, dwarf eggs, associated with the starting or the stopping of spawning, could be explained by a failure in the functioning of the albumen gland. In the case of the two eggs of *P. planorbis* that included 16 and 18 egg cells but no egg, the albumen gland may have ceased its production. The greatest number of egg cells per egg reached four for *P. planorbis* and 15 for *P. corneus.* This number was two for *A. fluviatilis* (Bondesen, 1950), six for *Physa fontinalis* (L.) (percentage of twins. 0.1–0.4%, De Witt, 1955), and 47 for *P. acuta* (Bigus, 1981). For the two species studied here, twins rarely hatched. However, six of the 15 embryos contained in the egg of *P. corneus* developed to early trochophore stage and moved energetically before dying.

The great majority of the capsules and eggs are normal. To what extent is the number of eggs per capsule a species feature? Does this number vary according to parent state (age, size) and environmental factors?

The planorbid family is characterized by the production of a smaller number of egg cells per egg in comparison with lymnaeids or physids. In relation to European planorbids, *P. corneus* has capsules rich in eggs. For example, Bloch (1938) observed 136 eggs in a single capsule. Here, maximum values of 48 egg cells per capsule in *P. corneus* and 46 in *P. planorbis* were seen. Moreover, in *P. corneus,* the mean number of eggs observed per mass was lower than the numbers found by other authors: 18.2 (Cole, 1925); 27.8 (N = 63) and 33.3 (N = 39) (Oldham, 1930); 71 (N = 28) (Alyakrinskaya, 1981); 15.6 or 33.3 according to respectively the small and the large race (Precht, 1936). In *Bithynia tentaculata* (L.), geographic provenance is the principal source of capsule-richness variation (Vincent & Gaucher, 1983). The number of eggs per capsule depends also on food (Van der Steen, 1967), dissolved oxygen in water (Alyakrinskaya, 1981), and crowding (Chernin & Michelson, 1957).

According to Oldham (1930), there does not appear to be a relationship between egg laying dates of *P. corneus* and the number of eggs per capsule. Here, however, the number of eggs per mass was generally low at the starting of spawning, and then increased. After this initial period, egg richness for *P. planorbis* was relatively constant, but was more variable for *P. corneus.* In the latter, a reduction in reproduction activity had a similar effect on the richness of capsules laid at 20°C, and an opposite result at 25°C. A negative correlation in *Lymnaea catascopium* (Say) (Pinel-Alloul & Magnin, 1979) and in *L. stagnalis* (Mooij-Vogelaar et al., 1970), or no correlation in *B. glabrata* (Vianey-Liaud, 1990) were found between the reproductive intensity and richness of masses in eggs. Unlike Vianey-Liaud (1990) for *B. glabrata,* but as Precht (1936) for *P. corneus* and Madsen et al. (1983) for *H. duryi,* Boag & Pearstone (1979) suggested that the biggest individuals of *L. stagnalis* laid the richest capsules. In our study, we did not notice a greater number of eggs per capsule over the growing period. In *L. fontinalis,* capsule richness was not proportional to snail size but depended on egg-laying date (Duncan, 1959). From field caged experiments, temporal measurements and dissections of females of the ovoviviparus prosobranch *Viviparus georgianus* (Lea), Buckley (1986) concluded: "spat size is positively correlated with female age irrespective of female size, though brood numbers increase with maternal size and growth rates."

A great variation of the capsule and egg production occurred for both species. Snails that produced a lot of capsules for two weeks should reduce their production after that, and we cannot envisage a lack of foreign sperm, because it could be stored in the spermatheca. A period of intensive reproduction was only observed for individuals of *P. corneus* reared at 20°C during the first half of their life, when their physiological state allowed them to reproduce. *Physa acuta* at 20°C showed a similar reproductive pattern (Perrin, 1986). Egg laying rhythms of 44 weeks (*P. corneus* at 15°C) and 56–58 weeks (*P. planorbis* at 10°C and 15°C) were observed using periodogram analysis. The rhythm of 44 weeks could be a multiple of a shorter rhythm (about 20 weeks according to Figure 1), and no obvious rhythm was found by the correlogram method (autocorrelation of time-series). Further experiments should be performed to confirm this rhythm and also the rhythms concerning *P. planorbis.* Moreover, it would be interesting to know if such rhythms are endogenous or not.

It is difficult to compare fecundities in different species because the reproductive function is strongly affected by experimental conditions, which are variable depending on authors. Nevertheless, some results are available: *B. glabrata:* 9,000 eggs and 7,000 newly hatched per year (Vianey-Liaud, 1990); *L. stagnalis:* between 4,655 and 10,832 eggs depending on light conditions and during a reproduction period of 7–13 months (Seuge & Bluzat, 1983); *H. duryi* during its whole life: between 145 (60 snails/2 water liters; reproduction period of 47 weeks) and 7,245 eggs (5 snails/2 l; reproduction period of 99 weeks) (Aboul-Ela & Beddiny, 1980). These three species are more fecund than the two studied planorbids: *P. corneus:* 1,600 newly hatched (20°C; 141 weeks), *P. planorbis:* 3,357 newly hatched (15°C; 125 weeks). Moreover, in both planorbids, the newly hatched snail production, which is continuous, increases with time until the penultimate year of life. In iteroparous mollusc species, reproduction effort increases with successive breeding seasons (Browne & Russell-Hunter, 1978). In the field, the fecundity of *Helisoma trivolvis* (Say) has been estimated at 1,962 eggs per adult snail during the spring breeding period, and at 1,263 eggs per snail in autumn (Eversole, 1978). Considering the tem-

perature for the minimum reproduction threshold (10°C for *P. planorbis,* 15°C for *P. corneus*) and the maximum numbers of eggs and newly hatched young produced, *P. planorbis* appears to require colder conditions than *P. corneus.* Such a result is also found for the optimum growth of these two species (Costil, 1994). From studies performed in seven species of aquatic snails, van der Schalie & Berry (1973) deduced that the lymnaeids reproduced and thrived best in cool (19°C to 22°C) conditions, whereas the planorbids required warmer water (22°C to 25°C), and the physids were highly tolerant, being able to maintain themselves in a much wider temperature range (12°C to 30°C). Our experimental approach is completed by studies performed in field (ecology, life cycle) (Costil, 1993), and all this research should allow us to get in the future a thoroughly knowledge of the biology of these two species.

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COLOUR POLYMORPHISM IN THE MANGROVE SNAIL *LITTORARIA INTERMEDIA* IN SINAI

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ABSTRACT

Samples of *Littoraria intermedia* Philippi (Gastropoda: Littorinidae) have been examined from an isolated mangrove at the southern end of the Sinai Peninsula, Egypt. The snails are abundant on the lower trunks and pneumatophores of *Avicennia marina. Littoraria intermedia* is usually monomorphic in shell colour, but here it is polymorphic. There is an orange morph, similar to the orange morph of leaf-living *Littoraria* species, at a frequency of about 8%, and there may also be distinct morphs in the dark class. The trunk substratum also shows distinct patches of brown and black colour. A substantial amount of predation by crabs is inferred from the observed frequency of shell breakage. Polymorphism may therefore occur in species of *Littoraria* in populations subject to predation. It is not, as has been suggested, restricted to situations where predation may be assumed to be negligible.

Key words: Littorinidae, predation, polymorphism, *Littoraria*.

INTRODUCTION

The genus *Littoraria* (Gastropoda: Littorinidae) consists of some 30 species associated with mangrove trees. Some of these live on bark surfaces and are usually monomorphic for shell colour; others live on the leaves of the trees and commonly exhibit shell-colour polymorphism (Reid, 1986). This association is sufficiently general for it to be interesting to examine exceptional cases. *Littoraria intermedia* is a very widespread species, extending from the east coast of Africa to the Pacific Ocean as far east as Hawaii, the Society Islands, and Samoa. Typically, it lives on roots and trunks of trees of the genera *Rhizophora* and *Avicennia.* Reid (1986) described the colour as "variable over the entire geographical range, but usually rather constant in each locality. Ground colour usually grey, sometimes pale brown, cream, whitish or rarely orange pink." He also noted (1986) that orange-pink shells are to be found in samples from Arnhem-land, Northern Territory, Australia, and (personal communication) from the Red Sea and Aqaba. Observations on the north coast of Papua New Guinea, in Thailand (Andaman Sea), and in Kenya indicated that the shells are uniform and very similar in colour in all these areas (Cook, 1986a, b; Cook & Garbett, 1992). On the other hand, Professor J. Heller (personal communication) suggested that the snails living on mangrove trees at a site on the Sinai Peninsula were polymorphic, in the sense of having clearly discontinuous phenotypes. From their locality, these should be *L. intermedia* (Reid, 1986), and we therefore decided to examine this colony in more detail.

THE MANGROVE SITE

The mangrove consists of stands of *Avicennia marina* (Forskål) on the coastal fan of the dry Wadi Kid. This is at the northern end of the Strait of Tiran, opposite the southern end of the Arabian side of the Gulf of Aqaba and a few km north of the village of Nabk. The site may be approached by traveling north along the coast from the airport that serves Sharm el Sheikh and the Na'ama Bay resort. The site is described in detail by Por et al. (1977). It consists of a series of lagoons and strands landward of a shallow fossil coral shelf, which is only covered by a few cm of water. The trees are short and have thick trunks, frequently damaged, with red-brown bark and dark brown to black areas where the wood has been exposed. Groves of pneumatophores extend from the trees to seaward and into the lagoons. Samples of snails were collected from pneumatophores and from trunks just above the water level at three locations: to the north, at the centre, and to the south of the curved end of Wadi

TABLE 1. Frequencies (%) of different colours in samples of shells of *Littoraria intermedia* from Wadi Kid, Sinai Peninsula. The standard error is for arcsin transformed frequencies.

Sample and location	Sample size	Orange	Grey/brown		
			dark	mid	light
1. North, trunk	232	7.5	30.1	57.3	5.0
2. North, trunk	297	7.8	24.5	61.6	6.1
3. North, pneumatophore	318	6.4	16.8	74.2	2.7
4. Central, pneumatophore	87	7.9	7.0	58.4	25.8
5. South, trunk	117	7.2	49.5	37.6	5.5
6. South, trunk	83	9.3	38.4	33.7	18.6
7. South, pneumatophore	74	8.2	14.9	56.8	20.3
Standard error		0.36	3.69	3.12	3.14

Kid. No snails were found on the leaves of the trees, and none of this species on rocks or stones.

COLOUR VARIATION AND FREQUENCY

Read (1986) records *Littoraria intermedia* from the Gulf of Aqaba as well as throughout the Red Sea. On the basis of shell shape and penis morphology, the mangrove littorinids collected at Wadi Kid belonged to this species. Three samples were taken from the north of the sequence, one from the centre, and three from the south. Three of these came from pneumatophores, and four from trunk surfaces (Table 1). This normally uniform species showed a considerable range of shell colour. As in other members of the genus, there appears to be a polymorphism for presence or absence of shell pigmentation and less clear-cut variation in the pattern of pigmentation, when present. In Table 1, four phenotypic categories are distinguished. The first column shows the frequency of shells with orange pigmentation. Most of these are bandless, but some have one or two grey-brown bands running along the whorls. The other three categories have the grey-brown pigmentation covering more or less the whole shell. These three groups vary in intensity of pigmentation from dark grey to a pale yellowish grey. Pigmentation is interrupted periodically as it is laid down during formation of the shell, so as to produce transverse pale and dark striping. The overall colour has two components, comprising the pigmentation and, where visible, the ground colour of the shell, which is yellowish. The colours of dry shells have been compared with standards in a colour handbook (Kornerup & Wanscher, 1967). This has a range of hues modified by tone and intensity, cross-referenced to names given to them in common English usage. The modal value for the category called orange here is between greyish-orange and greyish-red. The banding pigmentation is olive, and the yellow colour showing on banded shells is referred to as light yellow.

These categories show different amounts of variation across the samples, the frequency of orange being almost invariant whereas the grey-brown types vary between samples. For the comparison of orange and non-orange between samples $\chi^2 = 1.4$ with 6 degrees of freedom ($P > 0.9$). For the other three categories, $\chi^2 = 165.7$ with 12 degrees of freedom, which is highly significant. Another way of expressing this difference is to calculate the standard errors of the frequencies. When this was done using the arcsin transformed frequencies, the standard error for the grey-brown categories was found to be about ten times that of the orange category (Table 1). The implication is either that the environment affects the different morphs differently, or that it is not as easy to separate the grey-brown forms from each other as it is to distinguish them from orange. Variation in the grey-brown forms is not associated with position north to south or with bark or pneumatophore substratum.

In the species that live on foliage and are generally agreed to be polymorphic, a similar range of colour is found. There are yellow and orange forms, usually without bands but occasionally with narrow bands, and a category (dark), which is heavily banded. The orange forms in *L. pallescens, L. filosa, L. philippi-*

ana, L. luteola and *L. albicans,* illustrated by Reid (1986) in a colour plate, are the same colour as the orange class described here. The three categories are phenotypically distinct, and there is little difficulty in assigning shells to one or other of them, but variation in the banding patterns occurs. Thus, individuals of *L. pallescens* from Papua New Guinea show little variation within each morph, whereas those from Thailand show more variation (illustrated by Cook & Garbett, 1989). There seems no doubt that genetically controlled morphs are involved that affect both ground colour of the shell and nature and extent of banding. For practical descriptive purposes, this allows us to distinguish three forms in *L. pallescens:* yellow, orange, and dark. However, there is also variation in intensity of banding. Shells of the bark-living species *L. intermedia* and *L. scabra* are sometimes paler if the individuals are living on *Avicennia* than on the alternative preferred mangrove trees of the genus *Rhizophora* (Reid, 1986).

Given this information from other species and the pattern of variation in frequency between the samples of *L. intermedia* from Wadi Kid, we suggest that they are polymorphic for at least two forms, an orange morph analogous to, and possibly homologous with, the orange form of other *Littoraria* species, and a "dark" category. This is different from orange, possibly genetically heterogeneous and probably also subject to environmentally induced variation in expression.

EVIDENCE OF PREDATION

Damage arising from crab predation is an important source of mortality in tropical littoral molluscs (Hamilton, 1976; Vermeij, 1978, 1982, 1992). Many species of crab attack the shells from the mouth, breaking out crescentic pieces of shell that are likely to reflect the shape of the chela of the attacker. If the prey is not killed, it is likely to carry an irregular scar on the shell that provides evidence of past attack. Reid (1992) carried out an extensive study of the effect of crab predation on *Littoraria* species. Using exclusion arenas, he found direct evidence that the presence of predators is associated with the presence of scars on the shells of individuals that have survived attacks. High levels of damage occurred in *L. intermedia* and two other species living on bark but lower levels in two leaf-living species. There was also a higher level on *Rhizophora,* where there were more crabs, than on *Avicennia,* where there were fewer. Damage to shells in mangrove snails is much more likely to be due to predation than to accidental breakage (Vermeij, 1978), unlike intertidal species living in habitats where there is powerful wave action. Por et al. (1977) list 14 species of decapod Crustacea living in the area of the present study, some of which could be predators on the snails.

TABLE 2. Frequency of shells showing some breakage and repair in the samples, and mean shell height and shell strength estimated from sub-samples of 20 from each site. Strength is estimated as log load (N) required to break the shell divided by log shell height.

Sample	Damage (%)	Shell height (mm)	Log load/ Log height
1	28.9	17.2 ± 0.42	1.43 ± 0.011
2	28.9	18.1 ± 0.39	1.45 ± 0.010
3	15.1	17.4 ± 0.29	1.39 ± 0.019
4	19.4	18.8 ± 0.40	1.29 ± 0.020
5	20.6	14.0 ± 0.43	1.38 ± 0.018
6	12.3	15.7 ± 0.54	1.39 ± 0.020
7	7.1	15.7 ± 0.35	1.28 ± 0.014

Table 2 shows the frequencies of shells that show repaired cracks. There is variation between sites, but in some of the samples over a quarter of the shells are damaged. The heights of sub-samples of 20 shells from each site were measured to the nearest 0.1 mm with vernier callipers. The means and standard errors are given in Table 2. The load required just to break the shells was then established (measured in Newtons) using an Instron 4301 table testing machine. The method is described in Cook & Kenyon (1993). Table 2 also shows the mean and standard error for log load divided by log height. The means vary between sites ($F = 14.6$, d.f. 6 & 133, $P < 0.01$), the strongest being in the two northerly sites (samples 1 & 2) and the weakest in site 7. Shell size is greater in the first four sites than in the last three. The samples from the first two sites have the highest incidence of predation, whereas sample 7 has the lowest. There may therefore be a correspondence between attack and robustness of shell, which varies between different parts of the Wadi Kid mangrove.

DISCUSSION

Taken overall, there is a good association within the genus *Littoraria* of polymorphism with the foliage habitat and monomorphism with living on bark, on which the individuals are cryptic. There are two possible reasons for such an association. Either natural selection, probably through the agency of apostatic predation, selects for polymorphism on the vivid and heterogeneous background of foliage, whereas predation favours crypsis on bark (the selective hypothesis); or living on leaves removes species from the attention of predators to such an extent that variant forms may accumulate through mutation (the neutral hypothesis). Rosewater (1970) expressed the latter hypothesis as follows: "When snails leave the ground and ascend trees, they are immediately free of much of the danger from attacks by ground-living invertebrates and mammals which under ordinary conditions may select them for the familiar subdued coloration usually evidenced by many exposed land, freshwater and marine snails. It may be theorized, therefore, that in *L. scabra* [in which he included the leaf-living species] colour variation is not under the control of selective forces usually exerted upon other species of Littorinidae and is, therefore, freely expressed in many of its populations." It is not easy to decide between these alternatives. There appears to be good evidence for predation by crabs (Reid, 1992), which is sometimes heavy but is not necessarily selective. Reid (1987, 1992) has shown that apostatic selection, presumably through the agency of predation, may operate on leaf-living species. Some attempts to demonstrate selection have failed, however (Reid, 1987; Cook & Garbett, 1992), and a certain amount of selection does not in itself show that the *polymorphism* arises from selection. Provided the effective population is sufficiently large, a balance of mutation and accidental loss could be responsible (Cook, 1992). These species have a long-lived planktonic stage, so that large effective population size is possible.

One piece of evidence against the neutral hypothesis, although not a particularly strong one, is that different leaf-inhabiting species have similar morphs at similar frequencies. Darks are the most common (usually over 50%), yellows usually under 50% and orange morphs at 0–10% (Reid, 1986, 1987; Hughes & Jones, 1985; Cook, 1986a, 1992). Theoretically, this could come about if only a limited number of expressions of the genes concerned were possible and the morphs have the same relative mutation rates in the different species. However, the deterministic movement of gene frequency under mutation pressure is extremely slow. If we consider an allele at frequency q which mutates at rate u, and an alternative allele mutating at rate v, then the change in frequency per generation is $\Delta q = v - q(u+v)$. Integration gives an approximate expression for the number of generations, n, required for a given change in frequency. This is,

$$n = \ln\{[q_o(u+v)-v]/[q_n(u+v)-v]\}/(u+v)$$

If we start with the allele at frequency v, then the number of generations to get to 63% of the equilibrium frequency v/(u+v) is 1/(u+v), and to get within 100f% takes $-\ln(1-f)/(u+v)$ generations. Thus, the number of generations required to get near to equilibrium is several times the reciprocal of the mutation rates, probably millions of generations. Different species would be likely to exhibit different frequencies by chance.

The observations on the Sinai colonies constitute another, stronger, type of evidence. The argument suggests that bark-living species are usually monomorphic because selective predation favours crypsis on a uniform grey-brown background. Some species are very difficult to see, for example *L. strigata,* which has a disruptive transversely striated pattern making it inconspicuous on *Avicennia* trunks. Typically, *L. intermedia* is similar in colour and patterning to the background and does not stand out from it. No variant colours were seen, for example, among thousands of individuals examined on the north coast of Papua New Guinea. In the present instance, we have an isolated location in which the species is, exceptionally, polymorphic. The evidence indicates that it is also subject to predation and that robustness varies from sample to sample. The subjective impression is that the surface of the trees is broken into patches of bark and bare wood, which are much more distinct than usual and which have alternative grey and reddish colours like those of the morphs. It is possible to argue that, both here and in the case of foliage-living species, the heterogeneous background leads to selection for polymorphism (Cook, 1986b). At present, this is speculation. What is demonstrated here,

however, is that polymorphism does not occur only in the absence of predation, which is the neutralist conjecture.

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THE RELATIONSHIP BETWEEN SHELL-PATTERN FREQUENCY AND MICROHABITAT VARIATION IN THE INTERTIDAL PROSOBRANCH, *CLITHON OUALANIENSIS* (LESSON)

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ABSTRACT

A number of studies undertaken on the highly polymorphic intertidal mollusc *Clithon oualaniensis* reported that colour-morph frequencies varied on a regional basis in the Indo-Pacific region (Gruneberg, 1976, 1978, 1979). Our study examined colour-morph variation on a local scale in the same species and demonstrated that a level of variation similar to the regional variation described by Gruneberg was present in *Clithon* populations collected from different microhabitats at a single locality in northeastern Queensland. An examination of genetic differentiation (using allozyme electrophoresis) of the same populations failed to identify an association between genotype and microhabitat and confirmed that *Clithon* populations at least on a local scale belong to a single gene pool. Factors that influence the distribution of morphs at particular sites are most likely to be such ecological factors as differential predation. The results of this study indicate that relationships between environmental variables on a regional scale and colour-morph frequencies in *Clithon* need to be reassessed and the extent of local variation studied intensively.

INTRODUCTION

Polymorphisms provide opportunities to investigate evolutionary events in natural populations (Gillespie & Tabashnik, 1990), and some understanding of selective pressures and the maintenance of variability is possible (Reimchen, 1979). Colour polymorphisms have been investigated in many animals, including insects (Brakefield, 1990), birds (Hughes, 1982), fish (Endler, 1988), and mammals (Kettlewell, 1973).

In many mollusc species that show shell colour and pattern polymorphisms, a relationship between shell colour/pattern and environmental elements has frequently been suggested in the maintenance of the polymorphisms (Etter, 1988, *Nucella* sp.; Greenwood, 1992, *Cepaea* sp.; Chang & Emlen, 1993, *Cepaea* sp.). Differential predation has been suggested as a factor maintaining polymorphisms in many such species (Cook, 1983, *Littorina* sp.; review in Cook, 1986; Hughes & Mather, 1986, *Littorina* sp.; Reid, 1987, *Littoraria* sp.; review in Cook & Kenyon, 1991), although such other factors as temperature tolerance and area effects have also been reported (Jones et al., 1973).

A particularly striking example of shell colour and pattern polymorphism in gastropods is provided by the intertidal prosobranch *Clithon oualaniensis* (Gruneberg, 1976, 1978, 1979; Goodhart, 1987). The variation in *C. oualaniensis* is complex, with many shell colours and a large number of different banding patterns present (Gruneberg, 1976, 1978, 1979).

Clithon are widely distributed in the Indo-Pacific region and are commonly found on muddy sand, stones or seagrass beds in the upper reaches of the tidal flats in sheltered localities often near mangroves, the inlets of lagoons or the mouths of rivulets (Gruneberg, 1976), where they feed non-selectively on deposits (Dye & Lasiak, 1987) and are suspected of burying in the substrate across the high tide. Sometimes populations can be very large, and hundreds of snails may be found per square metre of substratum. The sexes are separate, and it is unlikely that *Clithon* has a free-swimming pelagic larva (Gruneberg, 1976), although information on the presence or absence of a larval phase is sketchy despite breeding trials having been attempted (Gruneberg, 1976).

In a number of studies, Gruneberg de-

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scribed shell colour and pattern polymorphisms in *C. oualaniensis* over a large geographic area of the Indo-Pacific (Gruneberg, 1976, 1978, 1979, 1982). Two regions were investigated: (a) populations from Ceylon and India ("western" *Clithon*); and (b) populations around Hong Kong and Malaysia ("eastern" *Clithon*). Gruneberg recognized snails from these two regions as being distinct "forms" of *Clithon,* found homogeneity with respect to the morph frequencies present within each of these regions, and suggested that this was equivalent to the "area effects" reported in the land snail *Cepaea nemoralis* by Cain & Currey (1963).

However, Gruneberg generally collected only one sample from a single locality (beach), assuming it to be representative of the whole beach, and, although he recorded the substratum type, he did not consider the effects of within-locality habitat variability. Temporal variation in morph frequencies at the one site (other than a comparison between juvenile and adult animals from the same collection) also was not investigated, although some studies of other polymorphic molluscs have shown morph frequency changes over time (Hughes & Jones, 1985, *Littorina* sp.; Hughes & Mather, 1986, *Littorina* sp.; Greenwood, 1992, *Cepaea* sp.).

The morph categories that Gruneberg described may have led to his reporting relatively uniform morph frequencies over large distances, because little attention was paid to the overall appearance of individuals and a great deal of attention was paid to relatively small differences in shell pattern that may have no selective advantage (assuming visual predation). In addition, Gruneberg's approach to statistical validation of the data was unusual because he compared frequencies of single morphs separately between regions, perhaps obscuring overall morph-frequency patterns between populations, instead of comparing frequencies of all morphs present in a population at the one time as is the more common approach (e.g. Reid, 1987).

Studies of other polymorphic gastropods have found variation in morph frequencies at a much smaller scale than those described by Gruneberg for *C. oualaniensis* (*Cepaea* sp.; reviewed in Cook, 1986). Observations of populations collected from single localities (beaches) have suggested that there can be considerable within-locality morph variation present, and where these differences exist they may relate to microhabitat differences (Cook, 1986; Hughes & Mather, 1986; Reid, 1987). If similar associations exist for *Clithon,* then Gruneberg's descriptions of between-region variation in morph frequencies need to be re-examined.

This study aimed to reassess the significance of regional morph variation in *Clithon oualaniensis* described by Gruneberg by determining whether there are shell-morph frequency differences within a single locality. Data from allozyme electrophoresis was also used to determine if cryptic species were present—as has been found in the genus *Littorina* (Mastro et al., 1982; Ward, 1990)—that may confuse the comparison of shell-morph frequencies. If the differences in morph frequencies among samples were due merely to chance and limited movement between areas, then significant differences in both shell-morph and allele frequencies at enzyme loci would be expected between sites. However, if the differences were due to differential removal of particular shell morphs by predators, then, as long as no linkage disequilibrium exists between allozyme and shell-pattern loci, the differentiation in morph structure should not be reflected in the allozymes.

MATERIALS AND METHODS

A study area was selected at Dingo Beach and two adjacent bays (Nellie and Champagne bays—approximately 20.8°S, 148.8°E) 40 km northeast of Proserpine, Queensland, Australia. The three localities showed similar microhabitat variation and had relatively high densities of *Clithon* that allowed for easy collection of large samples over short distances.

Selection of Microhabitats

Sampling was designed to assess the variability present within a single locality and variability among localities on similar microhabitat types.

Three distinct microhabitat types were chosen that represented the major microhabitats utilised by *Clithon* in the area:

1. seagrass—characterised by sand-mud substratum with a sparse covering of the seagrass *Halophila ovalis.*
2. rock/coral—fragments of dead coral, rocks and shells on a sand-mud substratum.
3. shelly sand—sandy substratum containing fine shell fragments.

TABLE 1. The location of populations, microhabitat types and sample sizes for all populations.

Population number	Time of collection	Locality	Microhabitat type	Sample size (N)
1	Feb. 1992	Dingo Beach	shelly sand	162
2	July 1992	Dingo Beach	shelly sand (0 m rep)	207
3	July 1992	Dingo Beach	shelly sand (15 m rep)	250
4	July 1992	Dingo Beach	shelly sand (30 m rep)	221
5	July 1992	Dingo Beach	shelly sand (45 m rep)	223
6	July 1992	Nellie Bay	shelly sand	248
7	July 1992	Champagne Bay	shelly sand	287
8	Feb. 1992	Dingo Beach	rock/coral	435
9	July 1992	Dingo Beach	rock/coral	263
10	July 1992	Nellie Bay	rock/coral	200
11	Feb. 1992	Dingo Beach	seagrass	187
12	July 1992	Dingo Beach	seagrass	244

The microhabitats consisted of a number of adjacent patches of approximately 100 to 150m^2, and beaches were a mosaic of different-sized patches of the various microhabitats.

The seagrass microhabitat was available on Dingo Beach only. Rock/coral microhabitats were sampled on Dingo Beach and Nellie Bay, and shelly sand microhabitats were sampled on Dingo Beach, Nellie Bay and Champagne Bay. Detailed sampling of the shelly sand microhabitat was undertaken at Dingo Beach in February and July 1992 to assess spatial and temporal variation within individual microhabitats.

Preliminary breeding studies on *Clithon* had shown direct genetic segregation of several characters (Gruneberg, 1976), although shell pattern is presumably under polygenic control (Goodhart, 1987). Following conventions outlined in Cain (1988) the term "form" will be used to describe different shell appearances (Gruneberg used "morph") because the specific characters in the combinations used have not been tested for heritability.

Population Sampling

Snails were collected in February and July 1992 (Table 1), with populations collected at both times taken from the same position in the microhabitat. All snails within an area defined by four 1 m^2 quadrats dropped randomly in a microhabitat were collected. Care was taken to collect all animals found within the quadrats to avoid selection only of the most conspicuous forms. It was assumed that this collection would be representative of the population at a particular site. All snails from one population were collected during a single low tide. Individuals were placed in numbered cryogenic vials while still alive and stored in liquid nitrogen until they reached the laboratory, where they were transferred to a −80°C freezer and stored pending scoring of forms and electrophoretic analysis.

Shell-Form Classifications

The basic pattern of *Clithon* consists of fine transverse ("axial") black lines on a coloured background (Gruneberg, 1976). This pattern is often complicated by the presence of triangles or "tongues." The size of these tongues is variable, as is the distance between the lines, although it is usually conserved within a single individual. A spiral pattern ("ladders") may be superimposed on the transverse lines exposing the background colour, and this region may contain tongues in "whorls" (Gruneberg, 1976). Usually there are three spirals, the thickness of which can vary, but some individuals may have only one thick central spiral. Variation in background colour ranges from white to green to deep orange. Occasionally, two background colours (in sharp zones like those of the spirals) may be present on a single individual. The pattern colour may be black or reddish pur-

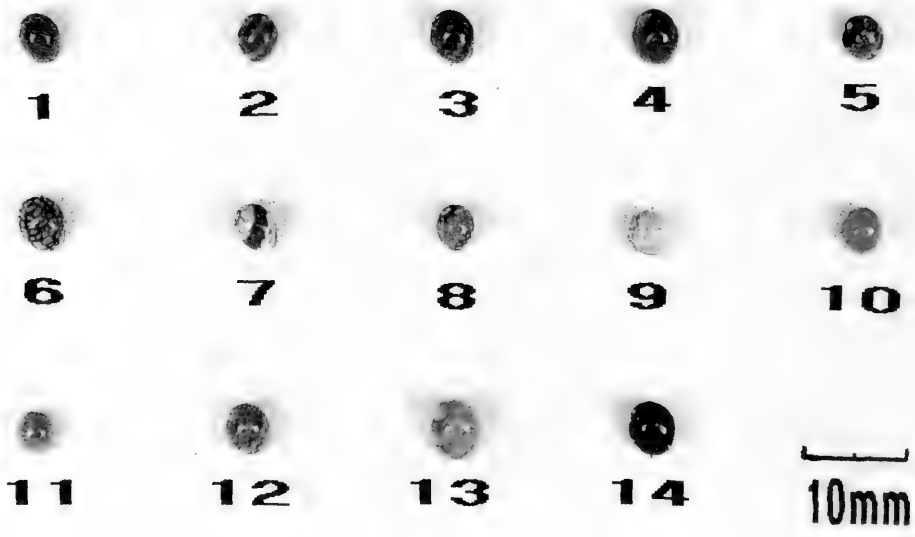

FIG. 1. Shell-form categories for *Clithon oualaniensis*.

ple. In addition, a sharp, distinct character may be present, referred to as a "purple spiral" by Gruneberg (1976). This character was only found at a zone near the suture and at the opposite end to the suture.

Individuals were scored for shell colour and pattern using a system modified from Gruneberg (1976). Juvenile shell patterns were scored using a stereo microscope, and when individuals were difficult to assign to specific forms they were excluded from the analyses. Gruneberg developed a system that was weighted heavily towards the type of patterning present on the shells, with a total of 17 main forms identified (Gruneberg, 1976). Our scoring method simplified the patterning emphasis by grouping similar categories. The difference between fine transverse lines and coarse transverse lines (Gruneberg, 1976) was considered to be of little significance, and these two forms were subsequently grouped. Similarly, "zebras" and "tigers" have been pooled, and narrow "spirals" and "spiral tongues" have been included with "ladders." Our approach involved a shift of focus from an emphasis on pattern type to a system combining pattern colour, shell colour and pattern type, which emphased the overall appearance of snails.

Snails that had a similar overall appearance were grouped into forms, with 14 forms being recognised. The smaller number of forms recognised in the present study would, presumably, mask differences that would have been present in Gruneberg's study, and if differences are found, they would be of a greater magnitude than those found by Gruneberg. The *Clithon* forms are shown in Figure 1 and described in Table 2.

Electrophoretic Analysis

Individuals were placed on ice immediately after removal from the freezer and were removed from their shells using forceps and a probe. Tissues were homogenised in 30 to 250 micro-litres of grinding buffer (Tris HCl pH 8 diluted 1:20 with distilled water plus 0.1% Triton X-100) (Richardson et al., 1986), depending on individual size. The homogenate was centrifuged at 6,000 rpm for 15 min at 4°C. The supernatant was removed and used immediately for electrophoresis on cellulose acetate plates (Titan III Zip Zone Cellulose Acetate Plates, Helena Laboratories, Texas USA) using a 75mM Tris citrate buffer pH 7.0 as an electrode buffer. The plates were then stained for the appropriate enzyme following

TABLE 2. Description of *Clithon oualaniensis* forms at Dingo Beach.

Morph No.	Description
form 1	characterised by closely spaced transverse lines with or without small tongues on a white or green background.
form 2	has 3 spirals (the pattern colour of which is black) superimposed on the basic pattern with a green background and usually tongues in whorls.
form 3	similar to Form 2 but with a red or purple pattern colour.
form 4	relate to what Gruneberg (1976) called 'tigers' and 'zebras'. They have widely spaced transverse lines on a green background with little or no tongues.
form 5	may be any pattern on a green background but must have a purple spiral.
form 6	similar to Form 4 but with large tongues covering the shell.
form 7	a single large spiral is found in these snails and the background within this spiral is usually green with the rest of the background white. Tongues in whorls may be present.
form 8	purple spiral on an orange or yellow background.
form 9	plain white or green snails with little or no pattern.
form 10	plain yellow or orange snails with little or no patterning.
form 11	yellow or orange snails with closely spaced transverse lines with or without small tongues.
form 12	tiger or zebra with or without tongues on a yellow or orange background.
form 13	spiralled orange snails usually found without tongues in whorls.
form 14	jet black appearance. Actually this form type has very close, black transverse lines on a whitish green (never yellow or orange) background.

procedures outlined in Richardson et al. (1986). Electrophoresis was performed at 4°C.

From each sample, individuals were examined for four polymorphic enzyme systems, representing six polymorphic gene loci: aspartate aminotransferase (E.C.2.6.1.1; Aat-1 and Aat-2 loci); esterase D (E.C.3.1.1.1; EstD-1); isocitrate dehydrogenase (E.C.1.1.1.42; Idh-1 and Idh-2) and 6-phosphogluconate dehydrogenase (E.C.1.1.1.44; 6pgd-1). Other polymorphic enzymes, including aconitase, adenylate kinase, and phosphoglucomutase, were not scored due to poor resolution, the enzymes denaturing after prolonged periods of freezing. For enzymes with more than a single locus, the most anodal locus was designated as 1, whereas alleles at individual loci were designated with alphabetic characters from the anodal (a) to the most cathodal (z). Alleles d and e at the EstD-1 locus were grouped as the d allele as they had very similar mobilities, and they were difficult to resolve on some plates.

Analysis of Genetic Data

Except where indicated, the computer program BIOSYS-1 (Swofford & Selander, 1989) was used to analyse allozyme data. The goodness of fit of observed genotype frequencies was tested to those expected if the population was in Hardy-Weinberg equilibrium using a χ^2 test. Deviation of observed genotype frequencies from expected values was estimated by the F_{is} statistic (Wright, 1965), in which $F_{is} = 1 - (H/2Np_iq_i)$ where H is the observed frequency of heterozygotes in the sample and N is the sample size.

Shell Form and Allelic Frequency Comparisons (Using a χ^2 Test for Independence)

Frequencies of shell form and allelic variation were compared between sites within one microhabitat, between like microhabitats from different localities, between different microhabitats, and over time within one habitat. To ensure expected values were ≥5, it was necessary to group some forms in some analyses. All groupings were based on similarity of appearance. The same problem arose with electrophoretic data, and genotypes were pooled into two to three classes when three or more alleles were observed at a single locus. Allelic frequencies at the Aat-1 and Aat-2 loci could not to be compared due to the near fixation of alleles observed at these loci.

RESULTS

Within-Habitat Variation

There was no significant variation in form frequencies between the four replicate samples taken from different areas of the shelly

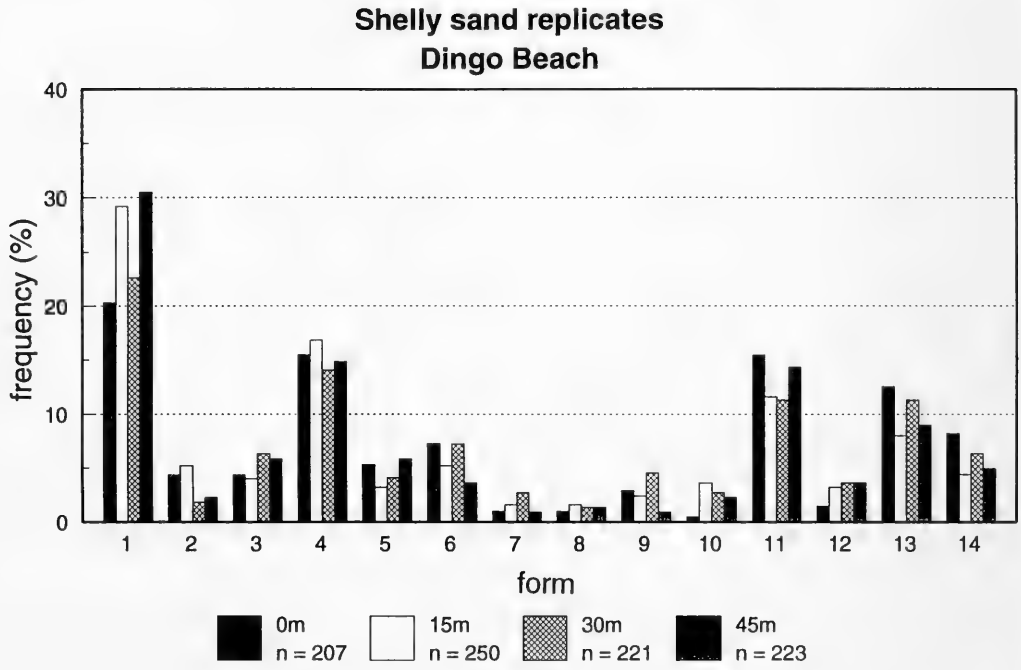

FIG. 2. Comparison of shell-form frequencies at four replicate sites in a shelly sand site.

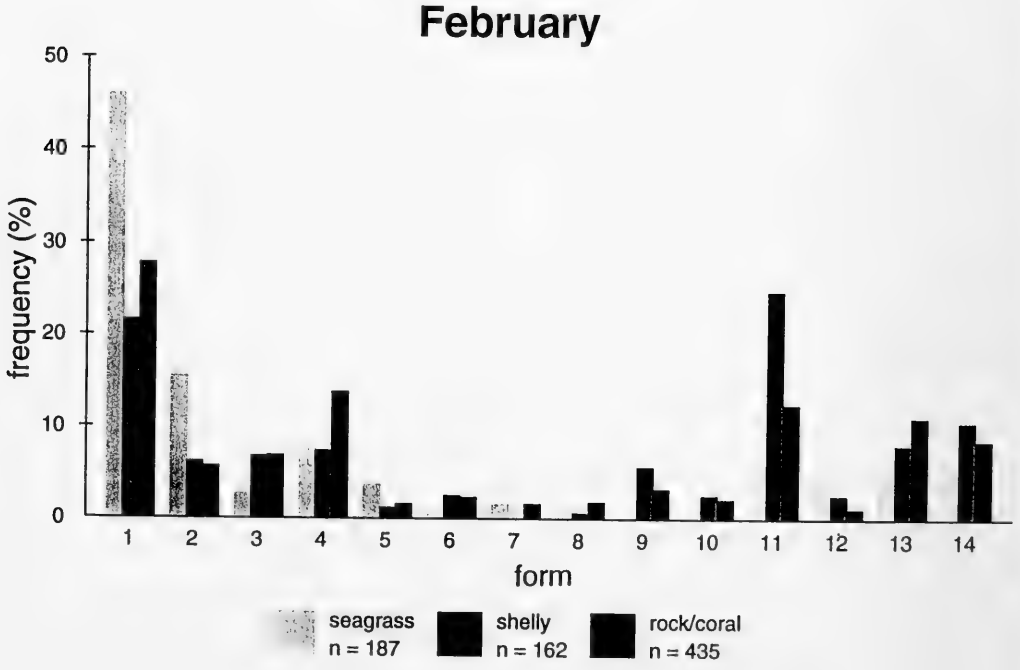

FIG. 3. Shell-form frequencies in three microhabitats in February.

sand microhabitat at Dingo Beach during July (Fig 2: $X_{39^2} = 44.2$, $p > 0.05$). The replicate samples from this analysis were thus considered as a single sample in further analyses.

Variation at a Local Scale

Analysis of the Dingo Beach data indicated significant differences in shell-form frequencies between micro habitats in both months (Fig 3: February $\chi_{20^2} = 98.7$, $p < 0.01$; Fig 4: July $\chi_{26^2} = 50.0$, $p < 0.01$). (Due to low expected values in some cells, forms 8 and 13, 10 and 11, and 5 and 6 were grouped for the Feb. analysis.) In the February collection, for example, the relative frequencies of forms 3, 11, 13 and 14 were lower, and forms 1 and 2 were a higher in the seagrass microhabitats than in other microhabitats. Forms 2 and 7 had higher relative frequencies in the seagrass microhabitat in the July collection, and the relative frequencies of forms 4, 11 and 13 were lower in the seagrass locality for the same month.

Temporal Variation in Shell-Form Frequency

There was significant temporal variation in form frequencies in the shelly sand and seagrass microhabitats (shelly sand $\chi_{12^2} = 35.3$, $p < 0.01$; seagrass $\chi_{12^2} = 29.9$, $p < 0.01$: forms 8 and 13 were grouped), but not in the rock/coral microhabitat ($\chi_{13^2} = 19.2$, $p > 0.05$) (Figs. 3, 4).

Temporal effects were most evident in the shelly sand microhabitat, where the relative frequencies of forms 1 and 4 had increased and the relative frequencies of forms 11 and 14 had declined between collection dates. Variation to a lesser extent was evident at the seagrass site, where a decrease in the relative frequency of form 1 occurred over time, while the frequency of most other forms (except form 2) increased. The non-significant result for different collection times at the rock/coral locality suggested higher temporal stability at this site.

Within Habitat Variation Among Localities

There was no significant variation in form frequencies between like microhabitats at different localities (Fig 5: shelly sand $X_{26^2} = 26.8$, $p > 0.05$; Fig 6: coral/rock $X_{11^2} = 12.5$, $p > 0.05$: forms 8 and 13, 10 and 11 were grouped). Therefore, the relative frequencies of shell forms did not vary between the same microhabitats (shelly sand and rock/coral) at different localities in July 1992.

Presence of Cryptic Species

If cryptic species were present, deficiencies of heterozygotes would be expected at sites where two or more species were represented in significant proportions. Thus, at some sites heterozygote defficiencies would be expected across the range of loci, whereas at other sites, where a single species comprised most of the sample, no significant effect would be detected. In addition, at those sites where low heterozygosity occurred, linkage disequilibrium would be expected.

Allele frequencies for all loci investigated in each population are shown in Table 3. The F_{is} values for populations that deviated from Hardy-Weinberg equilibrium are shown in Table 4. Some significant values were observed, but these are unlikely to be due to the presence of cryptic species because there was no consistency within sites. At only one site was more than one locus out of Hardy-Weinberg equilibrium, and in this instance only two loci out of six possible loci showed this result.

Polymorphic loci examined in this study were tested for linkage disequilibrium and no significant results were obtained.

Genetic Differentiation Among Sampled Populations

There was a significant difference in allelic frequency at the 6pgd-2 locus between populations on Dingo Beach collected in February (6pgd-2:$\chi_{4^2} = 19.458$, $p < 0.01$), but no significant difference was found in the July collections at this locus (6pgd-2:$\chi_{4^2} = 3.965$, $p > 0.05$). No significant differences were found at any other loci in either the February or July collections (February: Idh-1:$\chi_{2^2} = 0.339$, $p > 0.05$; Idh-2:$\chi_{2^2} = 0.55$, $p > 0.05$; EstD-1:$\chi_{6^2} = 3.331$, $p > 0.05$) (July: Idh-1:$\chi_{2^2} = 1.226$, $p > 0.05$; Idh-2:$\chi_{2^2} = 1.278$, $p > 0.05$; EstD-1:$\chi_{6^2} = 7.247$, $p > 0.05$).

There were no significant differences in allelic frequencies for any locus (Idh-1:$\chi_{3^2} = 3.601$, $p > 0.05$; Idh-2:$\chi_{3^2} = 2.6$, $p > 0.05$; EstD-1:$\chi_{9^2} = 6.953$, $p > 0.05$; 6pdg-2:$\chi_{6^2} = 3.57$, $p > 0.05$), indicating that allelic frequencies are consistent within and between microhabitats and that the Dingo Beach shelly

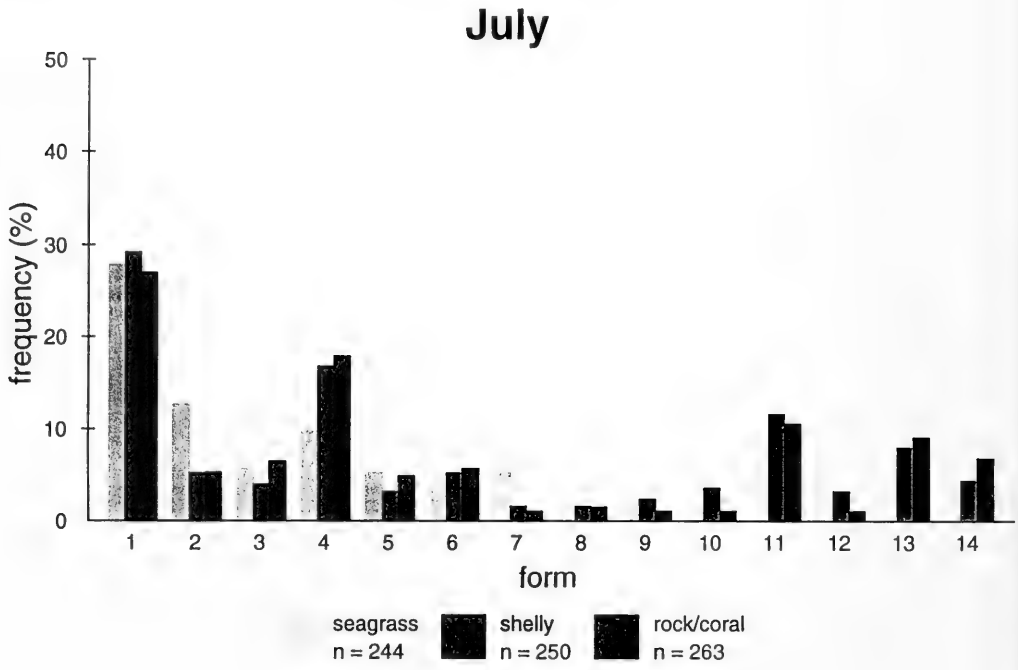

FIG. 4. Shell-form frequencies in three microhabitats in July.

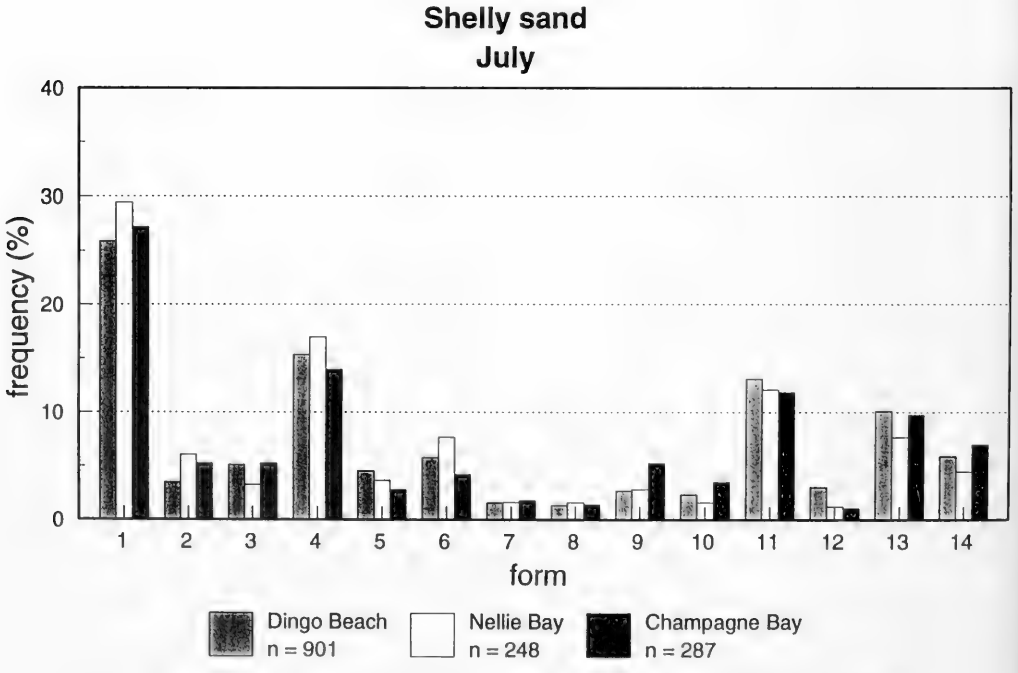

FIG. 5. Relative shell-form frequencies in shelly sand microhabitats at three localities.

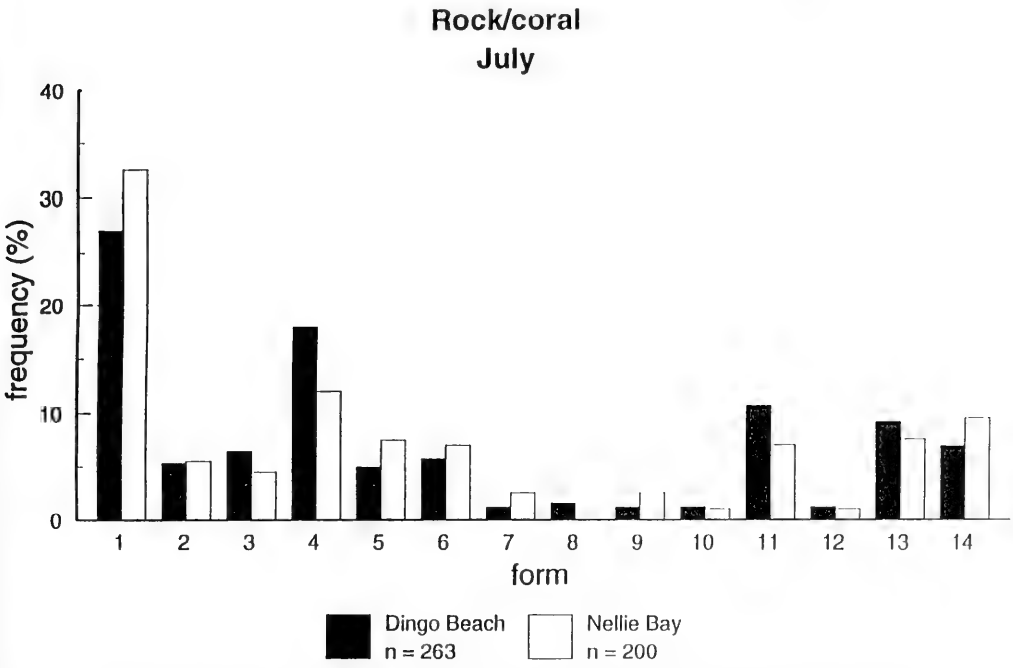

FIG. 6. Relative shell-form frequencies in coral/rock microhabitats at two localities.

sand populations (July 1992) can be considered as a single population.

Gene Flow Between Populations

Table 5 shows the results of Fst estimates for each locus in the sampled populations. Values are very low, ranging from 0.011 to 0.018, with a mean of 0.016, indicating that gene flow is extensive between microhabitats at the same locality and between adjacent localities. This lack of genetic differentiation indicated that the significant differences in form frequencies described earlier are not the result of limited gene exchange between populations occupying different microhabitats but must be due to other factors.

DISCUSSION

No evidence for the presence of cryptic species was obtained in this study, but significant differences in shell-form frequencies related to microhabitat were evident in the intertidal snail *Clithon oualaniensis* within a single locality (beach). Shell-form frequencies were also found to change temporally in some microhabitats. Differences in form frequencies at a local scale have not been previously reported for this species. There was, however, no evidence for a relationship between genotype and microhabitat utilisation in the enzyme loci investigated in this study, and any small differences in allelic frequency among sampled populations possibly reflects the lack of a larval dispersal phase (Gruneberg, 1976) or a sampling error due to the number of tests performed (Type I error). The lack of genetic differentiation among populations suggests a high level of gene flow. At any rate, the differences between sites in shell-form frequencies are much greater than any observed at allozyme loci.

Evidence of microhabitat and temporal differences in colour-form frequencies at a single locality requires a reassessment of Gruneberg's results (Gruneberg, 1976, 1978, 1979). Gruneberg (1979) compared populations from different geographic regions, including northeastern Queensland, Malaya-Singapore, Hong Kong, Bay of Bengal, and the Gulf of Mannar (Arabian Sea), and described significant differences in the frequencies of some forms between these areas. For

TABLE 3. Allelic frequencies for all populations.

	Population											
Locus	1	2	3	4	5	6	7	8	9	10	11	12
Idh-1												
(N)	22	47	45	48	48	48	47	72	59	48	58	45
A	0.00	0.01	0.00	0.01	0.01	0.03	0.00	0.01	0.01	0.00	0.01	0.01
B	0.86	0.85	0.87	0.82	0.71	0.79	0.77	0.85	0.80	0.85	0.82	0.84
C	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
D*	0.14	0.13	0.13	0.17	0.27	0.17	0.23	0.14	0.20	0.13	0.17	0.14
E	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00
Idh-2												
(N)	22	45	45	48	48	48	48	72	59	48	57	45
A	0.91	0.91	0.89	0.96	0.96	0.93	0.89	0.87	0.94	0.93	0.91	0.92
B	0.09	0.09	0.11	0.04	0.02	0.07	0.12	0.10	0.06	0.07	0.09	0.08
C	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
Aat-1												
(N)	32	48	48	48	48	48	48	72	60	48	60	46
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
B	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.00
C	0.98	0.98	0.99	1.00	0.98	1.00	1.00	1.00	1.00	0.98	1.00	0.98
D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Aat-2												
(N)	32	48	48	48	48	48	48	72	60	48	60	46
A	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.01	0.00	0.01
B	0.00	0.00	0.03	0.00	0.02	0.01	0.00	0.01	0.02	0.02	0.02	0.01
C	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.01
D	0.98	0.95	0.96	1.00	0.97	0.95	1.00	0.97	0.97	0.96	0.96	0.97
E	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	0.02	0.00	0.01	0.00	0.00	0.02	0.00	0.02	0.02	0.00	0.03	0.00
6pgd-2												
(N)	33	46	46	48	48	48	48	72	58	46	59	45
A	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00
B	0.20	0.02	0.01	0.02	0.03	0.01	0.00	0.01	0.01	0.07	0.00	0.01
C	0.64	0.51	0.49	0.48	0.64	0.57	0.63	0.62	0.50	0.46	0.62	0.66
D	0.17	0.44	0.45	0.45	0.30	0.37	0.35	0.35	0.48	0.41	0.38	0.30
E	0.00	0.03	0.05	0.05	0.03	0.04	0.02	0.02	0.01	0.05	0.00	0.03
EstD-1												
(N)	12	47	44	47	47	48	46	69	59	46	36	45
A	0.08	0.12	0.26	0.12	0.14	0.16	0.11	0.12	0.14	0.13	0.18	0.08
B	0.71	0.59	0.46	0.66	0.56	0.58	0.55	0.49	0.49	0.69	0.49	0.62
C	0.13	0.20	0.16	0.15	0.18	0.09	0.23	0.23	0.23	0.13	0.17	0.19
D	0.08	0.10	0.13	0.08	0.12	0.17	0.11	0.17	0.14	0.05	0.17	0.11

*Represents a null allele at this locus

example, the form "purple spiral" was reported to have an incidence of 15.5% in northeastern Queensland as compared to 0.015% in the Gulf of Mannar. With no detailed investigation of form-frequency differences within local areas, however, Gruneberg could not know if similar differences were present on much smaller geographic scales. The present study has shown significant differences between different microhabitats on a local scale. Therefore, any inferences about frequency differences on larger scales without such information is open to question. Moreover, any correlation of form frequency with an environmental variable—e.g. surface salinity, as Gruneberg (1979) suggested—has to be viewed with caution unless the relationship can be shown to be related to local form-frequency differences as well.

Gruneberg's (1982) explanation for the variability in shell colour and pattern in *C. oualaniensis* as being "pseudo-polymorphic" has also been criticised by some au-

TABLE 4. Departure from Hardy-Weinberg as estimated by the fixation index.

Pop. number	Locus	Observed heterozygotes	Expected heterozygotes	Fixation index (F_i)
1	Idh-1	2	5.182	0.614
2	Idh-2	4	7.289	0.451
3	Aat-2	2	3.865	0.482
	Idh-2	4	8.889	0.550
4	EstD-1	20	24.670	0.189
5	Idh-2	2	3.875	0.484
12	Idh-1	9	11.967	0.248

thors (e.g., Goodhart, 1987). The delineation of "eastern" and "western" *Clithon* morph frequencies by Gruneberg (1976, 1979) was made on the basis that particular morphs occurred at significantly different frequencies between regions. For example, three different types of "ladders" were recognised by Gruneberg (1979): "spiral tongues" (tongues in whorls), "ladders proper" (spirals with no tongues), and "yellow spirals" (spiralled orange). He reported that the relative frequencies of each of these morph categories differed considerably from one population to another (in "western *Clithon*"). Gruneberg attributed this to the influence of environmental conditions that vary irregularly in time and space, and suggested that the "morphs" did not relate to separate genotypes. However, in Gruneberg's "eastern *Clithon,*" ladders were not observed, although yellow spirals and spiralled tongues were present (Gruneberg, 1979). He attributed these differences to a founder event, with the variation in the phenotypic expression of the ladder morphs in "western *Clithon*" absent in the eastern form. Thus, in one region all ladders were supposed to be the same genotype expressed differently due to environmental conditions, but in his eastern populations the different ladder morphs were supposed to be separate genotypes. All the morphs found in Gruneberg's eastern and western populations were found in the present study, and there seems little basis for his separation of regional (eastern vs. western) classes of *Clithon* populations.

Temporal variation in relative form frequencies found in the present study also suggests a further criticism of Gruneberg's interpretations. Temporal variation may be due to differences in mortality between forms in different microhabitats possibly caused by predation (Cook, 1986; Reid, 1987), variable temperature conditions (Cook, 1986; Greenwood, 1992) or variation in physiological stress (Etter, 1988) for example. The presence of temporal changes in shell-form frequency add support to our suggestion of some external factor affecting form frequencies and not subpopulation structuring as would be the case if cryptic species were present.

TABLE 5. Fst values for all loci for February and July samples.

LOCUS	FEB Fst	JULY Fst
Idh-1	0.002	0.015
Idh-2	0.003	0.011
Aat-1	0.010	0.011
Aat-2	0.003	0.011
6pgd-2	0.034	0.018
EstD-1	0.026	0.017
Mean	0.021	0.016

A number of alternative explanations have been proposed to explain shell-pattern polymorphisms in gastropod species. The importance of crypsis was stressed by Goodhart (1987), who considered that a generally cryptic appearance should be favoured by natural selection except in such special cases as warning colouration in many noxious creatures. Extremely polymorphic species (such as *Clithon*) may contain different morphs that are at an equal selective advantage in different microhabitats, with a range of morphs able to exist in all situations where the species is found (Endler, 1988). However, some morphs may be more favoured in certain microhabitats, which could lead to form-frequency differences as were found in this study. Thus, differences in form frequences on local and larger scales may be explained by a combination of frequency-dependent selection (Clark et al. , 1988) and selection for crypsis.

Predator-mediated selection for crypsis is

thought to be the most plausible explanation for the relationship between shell-colour-form frequencies and habitat in *Clithon.* However, detailed caging experiments, such as those of Hughes & Mather (1986), would be necessary before there is substantial evidence of selection for crypsis influencing the form frequencies of *Clithon* populations in different microhabitats. By eliminating all potential predators of this species and manipulating the form frequencies in caged and non-caged areas, the survival of various forms in different microhabitats could be assessed.

Therefore, before any explanations that seek to use environmental gradients and variables to explain form-frequency differences in shell-colour patterns in *Clithon oualaniensis* can be accepted, more detailed analyses of local variation in these characters needs to be undertaken. Our study would indicate that, at best, any positive correlations between environmental variables and colour-form frequencies on a regional scale may relate to the fact that local populations were collected from different microhabitat types from localities within each region. So, significant differences in form frequencies between regions perhaps reflect differences in microhabitat availability between the regions, and where the same microhabitats are available within an individual site, the same or related significant form-frequency differences may also exist.

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EL GÉNERO *CANARIELLA* HESSE, 1918, Y SU POSICIÓN EN LA FAMILIA HYGROMIIDAE (GASTROPODA, PULMONATA, HELICOIDEA)[1]

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ABSTRACT

Canariella is a poorly known genus of the Hygromiidae, endemic to the Canary Islands, with 18 nominal taxa of specific and subspecific rank. Until now, no information on the internal anatomy of its genital ducts was known, and the external morphology of the genital system, which lacks any trace of the dart-sac complex, was known for only five species.

In another article (Groh et al., in press), four nominal taxa (= three species) of *Canariella* were described conchologically and anatomically. The present work treats the remaining known species. (We exclude *Helix plutonia* Lowe, 1861, which has been included in *Canariella* but really belongs in a new genus of the Hygromiidae.)

Lectotypes are designated for the type species, *Carocolla hispidula* Lamarck, 1822; *Helix bertheloti* Férussac, 1835; *H. everia* Mabille, 1882; *H. fortunata* Shuttleworth, 1852; *H. (Gonostoma) hispidula subhispidula* Mousson, 1872; and *H. (Ciliella) lanosa* Mousson, 1872. The holotypes of *Helicodonta salteri* Gude, 1911, and *Helix (Gonostoma) beata* Wollaston, 1878, are also studied. These eight taxa differ slightly from one another in shell morphology, but agree in the morphology of the genital system, and there is no geographical isolation among them (Fig. 39); therefore, they are considered to belong to a single species, and the last seven names are synonymized with *Carocolla hispidula*. However, six populations are conchologically distinguishable, a sign of the beginning of radiation, and therefore we consider them with the rank of infrasubspecific varieties.

Lectotypes of *Helix (Gonostoma) gomerae* Wollaston, 1878; *Carocolla planaria* Lamarck, 1822; *Helix afficta* Férussac, 1832; and *H. eutropis* Shuttleworth, 1860, are also designated.

Applying the results of this study and the authors' knowledge of further new species not yet described, the following new diagnosis of *Canariella* is proposed:

Mantle collar with four small lobes (subpneumostomal, left dorsal, right dorsal and right lateral; as an exception, *C. eutropis* has also the left lateral lobe). Kidney sigmurethric, without secondary ureter. Central and first lateral radular teeth with small but evident ectocones. Right ommatophore retractor passing between penis and vagina. Genital system without the dart-sac complex and with several vaginal digitiform glands, each with an independent, very slender initial portion; they are crown-shaped when there are more than three. With a sheath surrounding the distal male duct (between the atrium and the penis retractor muscle insertion). With internal differentiation penis-epiphallus (externally this differentiation can be undistinguishable). Penis retractor muscle with an epiphallar insertion. Penial nerve originating from the right cerebral ganglion (verified in the type and two additional species).

Canariella is considered as "incertae sedis" within the Hygromiidae and is compared with several other hygromiid genera without the dart-sac complex. The most closely related genera are *Montserratina, Ciliella* (which is not present in the Canary Islands, in spite of published records), *Schileykiella, Tyrrheniellina* and *Ciliellopsis.* Other Hygromiidae species lack dart-sac complex, but they differ in the presence of vaginal appendages or in the morphology of the terminal parts of the male ducts.

Key Words: Pulmonata, Hygromiidae, *Canariella*, systematics, Canary Islands.

INTRODUCCIÓN

El género *Canariella* Hesse, 1918, endémico del Archipiélago Canario, es uno de los representantes de la familia Hygromiidae peor conocidos en la actualidad, a pesar de que los primeros datos bibliográficos existentes sobre sus especies se remontan a principios del siglo XIX. En efecto, Férussac (1821), publicó los nombres de las dos pri-

[1]Notes on the Malacofauna of the Canary Islands, No. 28 (subvencionado con el proyecto 92/160 del Gobierno de Canarias).

meras: *Helix (Helicigona) afficta* y *H. (Helicigona) lens,* siendo ambos nombres "no disponibles" (ICZN, Artículo 12). Al año siguiente, Lamarck (1822) describió ambas especies y las denominó *Carocolla planaria* y *Carocolla hispidula*, respectivamente. Desde entonces, se han descrito (la mayoría sólo conquiológicamente) y asignado a este género 18 taxones nominales del nivel especie:

1. *Carocolla hispidula* Lamarck, 1822
2. *Carocolla planaria* Lamarck, 1822
3. *Helix (Helicigona) afficta* Férussac, 1832
4. *Helix bertheloti* Férussac, 1835
5. *Helix leprosa* Shuttleworth, 1852a
6. *Helix fortunata* Shuttleworth, 1852a
7. *Helix discobolus* Shuttleworth, 1852b
8. *Helix eutropis* Shuttleworth, in Pfeiffer, 1860
9. *Helix (Macularia) plutonia* Lowe, 1861
10. *Helix (Ochthephyla) multigranosa* Mousson, 1872
11. *Helix (Ciliella) lanosa* Mousson, 1872
12. *Helix (Gonostoma) hispidula subhispidula* Mousson, 1872
13. *Helix (Gonostoma) gomerae* Wollaston, 1878
14. *Helix (Gonostoma) beata* Wollaston, 1878
15. *Helix everia* Mabille, 1882
16. *Helix pthonera* Mabille, 1883
17. *Helix (Gonostoma) parryi* Ponsonby y Sykes, 1894
18. *Helicodonta salteri* Gude, 1911

Únicamente se ha descrito la anatomía externa del aparato reproductor de cinco de ellos (números 1, 5, 6, 7 y 9), por Krause (1895), Hesse (1931) y Odhner (1931); y está en vías de publicación la redescripción de otros tres (números 10, 16 y 17, junto con una especie nueva para la ciencia: Groh et al., en prensa), incluyendo por primera vez datos de la anatomía interna de sus aparatos reproductores. Finalmente, está en vías de publicación la descripción de una nueva especie subfósil (Hutterer, en prensa) y la redescripción del número 9, que en realidad pertenece a un nuevo género de Hygromiidae.

El presente trabajo está dedicado a los restantes taxones nominales conocidos del género, entre los que se encuentra la especie tipo. En base a los resultados de este estudio y a los datos que poseemos de las otras especies existentes del género (todavía no descritas), en este artículo se corrige la diagnosis de *Canariella* y se discute su posición entre los Hygromiidae.

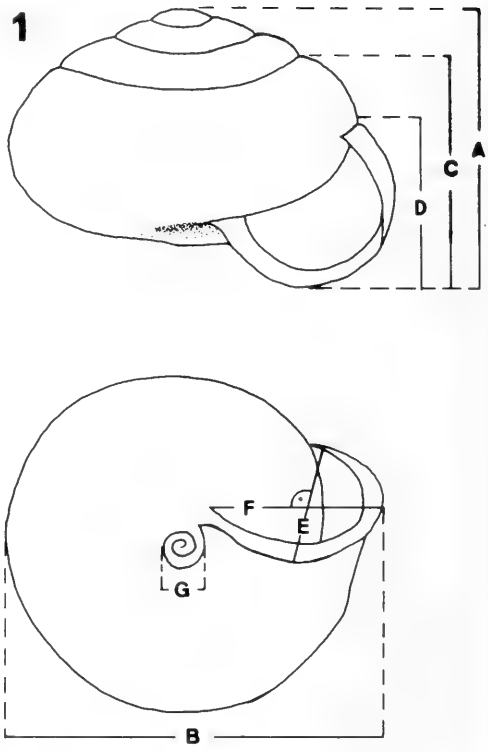


FIG. 1. Medidas tomadas en la concha (explicación, en el texto).

METODOLOGÍA

Para hacerlas más objetivas, las descripciones se han basado, en parte, en datos biométricos. Las medidas, realizadas con un calibrador digital electrónico conectado a una computadora, son las siguientes (Fig. 1): A: altura de la concha; B: diámetro de la concha; C: altura de la última vuelta de espira; D: altura del lado ventral de la concha; E: longitud de la abertura; F: anchura de la abertura; G: diámetro del ombligo (sin peristoma); H: altura del lado dorsal de la concha (= A−D). Con ellas se han calculado los valores máximo (M), mínimo (m) y medio (X), así como el coeficiente de variación (CV) de Pearson (expresado en %), indicándose en cada tabla el número de ejemplares medidos (n) del taxón correspondiente. Es necesario señalar que el diámetro del ombligo (G) es difícil de tomar en muchos casos, bien por sus pequeñas di-

mensiones, o porque el peristoma lo tapa parcial o incluso totalmente. Cualquier error por pequeño que sea o, simplemente, la existencia de variabilidad en sus dimensiones, es más apreciable que en las otras medidas, que son de mayor magnitud. Esto queda claramente reflejado en los altos valores que presenta el coeficiente de variación de Pearson en esta medida, por lo que ni ella ni el índice en el que participa son válidos para un estudio estadístico; sin embargo, sí lo son para dar información sobre su tamaño desde el punto de vista descriptivo.

Con estas medidas se han calculado los parámetros e índices que se indican a continuación. Los intervalos utilizados se han calculado teniendo en cuenta todas las especies del género.

— Tamaño de la concha (B). En función de su diámetro, la concha es:

pequeña:	< 11.50
mediana:	11.50 – 15.76
grande:	15.76 – 19.02
muy grande:	> 19.02

—Indice de la forma de la concha (A/B). Relaciona la altura de la concha con su diámetro. Según su valor, la concha es:

aplanado-lenticular:	< 0.485
deprimida:	0.485 – 0.60
cónico-ovalada:	> 0.60

—Indice de la forma dorsal (H/B). Relaciona la altura de la parte dorsal de la concha con su diámetro. Según su valor, la concha dorsalmente es:

aplanada:	< 0.225
cónica:	> 0.225

—Indice de la forma ventral (D/B). Relaciona la altura del lado ventral de la concha con su diámetro. Según su valor, la concha ventralmente es:

aplanada:	< 0.375
ovalada:	> 0.375

—Indice de la forma de la abertura (E/F). Relaciona la longitud de la abertura con su anchura. Según su valor, la abertura es:

ovalado-deprimida:	< 0.825
ovalada:	0.825 – 0.895
redondeada:	> 0.895

—Indice del tamaño del ombligo (B/G). Relaciona el diámetro de la concha con el del ombligo. Según su valor, el ombligo es:

muy pequeño:	> 29.095
pequeño:	20.57 – 29.095
mediano:	11.50 – 20.57
grande:	< 11.50

En cuanto al aparato reproductor, no se han tomado medidas de sus conductos al observar que existe variabilidad en sus dimensiones dentro de una misma especie. Esta variabilidad probablemente se debe al diferente momento del desarrollo del reproductor con respecto a la fase de reproducción. Además, en muchas especies el número de aparatos reproductores disponibles en estado adulto no era suficiente para que las medidas obtenidas fueran estadísticamente fiables. Con respecto a la terminología del aparato reproductor, se utiliza la palabra "proximal" para designar a la zona más cercana a la gónada, y "distal" para la más cercana al orificio genital.

Abreviaturas: ANSP— Academy of Natural Sciences, Philadelphia; CGH— K. Groh private collection, Hackenheim; DMNH— Delaware Museum of Natural History, Greenville, FMNH— Field Museum of Natural History, Chicago, MHNG— Muséum d'Histoire Naturelle, Genève; MNHN— Muséum National d'Histoire Naturelle, París; NHM— Natural History Museum, London; NMB— Naturhistorisches Museum, Bern; NMW— National Museum of Wales, Cardiff; TFMC— Museo de Ciencias Naturales de Tenerife, Santa Cruz de Tenerife; ZMZ— Zoologisches Museum der Universität, Zürich.

DESCRIPCIONES TAXONÓMICAS

Familia Hygromiidae Tryon, 1866

Género *Canariella* Hesse, 1918

Especie tipo: *Carocolla hispidula* Lamarck, 1822. Designación: Hesse (1918: 106–107).

Diagnosis Original

"An dem stark geschwollenen Penis sitzt ein schmächtigerer, nach dem Vas deferens zu sich verjüngender Epiphallus und ein winziges Flagellum mit hakenförmig umgebogener Spitze. Der Retractor ist an der Grenze von Penis und Epiphallus angeheftet; an der

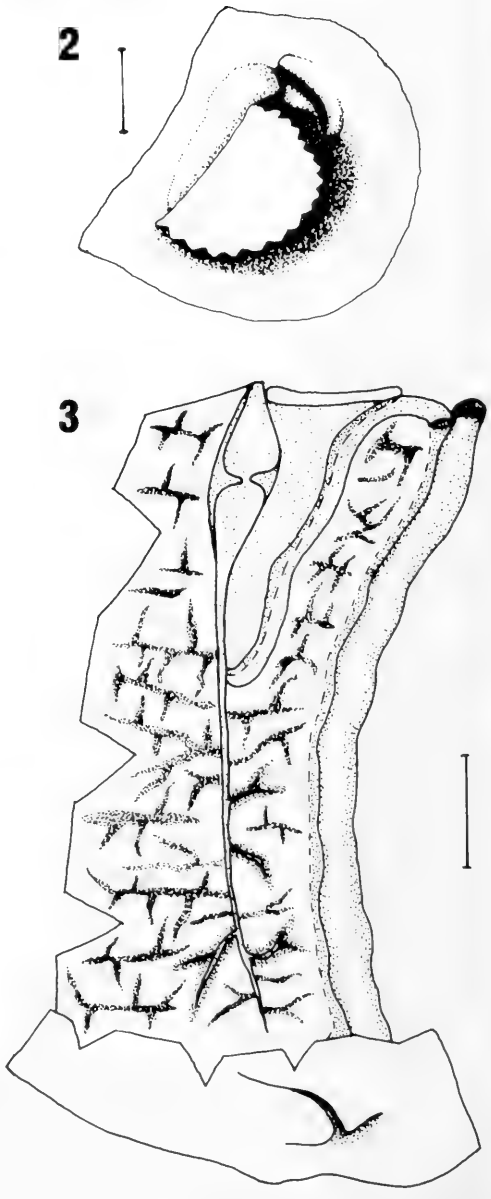
langen Vagina sitzen drei ziemlich lange, dünne, cylindrische Glandulae mucosae; Pfeilsack nicht vorhanden. Samenblase kugelig auf kräftigem Stiel. Uterushals etwa halb so lang wie die Vagina" (Hesse, 1918). [A continuación del pene, grueso, hay un delgado epifalo que, después del conducto deferente, se estrecha en un diminuto flagelo con la punta torcida a modo de gancho. El músculo retractor está sujeto en el límite entre el pene y el epifalo; en la vagina, larga, hay tres glándulas mucosas bastante largas, delgadas y cilíndricas; no existe saco del dardo. La bolsa copulatriz es esférica y está sobre un conducto grueso. El cuello del útero es casi la mitad de largo que la vagina.]

Hesse (1918) se basó únicamente en la forma externa del aparato reproductor, por lo que se equivocó al considerar que el límite entre el pene y epifalo está a nivel de la inserción del músculo retractor; en realidad, este músculo se inserta en el epifalo, hecho que fue reconocido posteriormente por este autor (Hesse, 1931) en *Helix fortunata.* Además, hay otras características importantes que pueden ser incluídas en la diagnosis; por ello, a continuación se realiza una breve descripción del género, y de ella se extrae una nueva diagnosis.

Descripción del Género *Canariella*

La concha mide de 6 a 21 mm de diámetro y tiene de 4 a 6 3/4 vueltas de espira; normalmente es aplanada, angulada o aquillada y está cubierta por pelos periostracales menores de 1 mm de largo. El color más típico es marrón claro y la ornamentación de la teloconcha está formada por costulaciones radiales en número y grosor variables, sobre las que se superponen crestas espirales muy finas. El peristoma no está engrosado y forma un pequeño labio en las zonas columelar y basal; sus extremos normalmente se insertan alejados entre sí en la zona parietal y entre ellos, en los individuos más viejos, hay una zona provista de una tenue callosidad.

El collar del manto (Fig. 2) tiene forma de "D" inclinada y el ángulo superior se encuentra cerca del pneumostoma, en cuyas proximidades se disponen cuatro lóbulos poco desarrollados (la nomenclatura está basada en Gittenberger & Winter, 1985). El lateral derecho es el más largo y grueso; es relativamente ancho en la zona de contacto con el ano y adelgaza progresivamente hasta la parte media del collar. El dorsal derecho está situado encima del ano y es muy pequeño, casi inconspicuo. El dorsal izquierdo es más conspicuo que el derecho, terminando en un extremo prominente. El subpneumostomal tiene forma triangular y está apenas engrosado. Salvo en *C. eutropis,* es destacable la ausencia del lóbulo lateral izquierdo, que está presente en otros Hygromiidae.



FIGS. 2–3. *Canariella hispidula* var. *hispidula* (Tabaiba Alta, Tenerife). (2) Collar del manto. (3) Complejo paleal. Escala: 1 mm.

El complejo paleal (Fig. 3) ocupa la última vuelta de espira. El techo del pulmón presenta una serie de manchas oscuras, irregulares. El riñón es sigmurétrico, sin uréter secundario, la mitad de largo que el pulmón y el doble que el corazón.

La mandíbula (Fig. 18), odontognata, está provista de un número variable de costillas, que pueden ser anchas o estrechas, salvo en los laterales, donde es casi lisa. La rádula (Figs. 19, 20) consta de 90 a 140 filas de dientes, sin que se aprecie delimitación clara entre los laterales y los marginales. El central tiene un mesocono de punta ligeramente aguda y dos ectoconos pequeños, pero nítidos, en su base. Los primeros dientes laterales son más grandes y robustos que el central y están provistos de un mesocono y de un pequeñco ectocono, ambos puntiagudos. Hacia los márgenes, la anchura del mesocono disminuye a la vez que aumenta la del ectocono, que puede tener su cúspide dividida en dentículos.

Aparato reproductor (Figs. 28–38). El atrio es corto. El músculo retractor del pene se inserta en el epifalo. El pene es tubular y se diferencia del epifalo por su anatomía interna (externamente esta diferenciación puede no apreciarse); está envuelto por una vaina fina y traslúcida, que se suelda a él en su extremo distal (junto al atrio) y al epifalo a nivel de la inserción del músculo retractor. El conducto deferente desemboca lateroapicalmente en el epifalo, junto con el flagelo. El epifalo es tubular y alberga en su interior dos a cinco pliegues longitudinales, de los que generalmente se prolongan dos en la cavidad del pene, fusionándose parcialmente entre sí y formando una papila peneana acanalada. La papila en algunas especies ocupa poco espacio en la luz del pene (Figs. 28–35c), o incluso puede faltar completamente, como en *Canariella multigranosa* (Mousson, 1872; Groh et al., en prensa), mientras que en otras llega a ser muy grande (Figs. 37–38c), separando la cavidad del epifalo de la del pene. Éste posee además, en su interior, una serie de boceles longitudinales. La vagina es tubular y también está provista internamente de boceles longitudinales. Carece de saco del dardo, sacos accesorios y apéndices vaginales accesorios. En ella desembocan 1–8 glándulas vaginales digitiformes, normalmente simples y dispuestas, cuando hay varias, formando una corona, cuyo diámetro se estrecha bruscamente en la base, antes de su unión con la vagina. El conducto de la bolsa copulatriz alberga gran número de pliegues irregulares.

El músculo retractor del ommatóforo derecho pasa entre el pene y la vagina. El nervio peneano aparentemente se origina del ganglio cerebroideo derecho (hasta ahora sólo ha sido confirmado en tres especies, entre ellas la especie tipo del género).

Nueva Diagnosis del Género *Canariella*

Collar del manto típicamente con cuatro lóbulos poco desarrollados (lateral derecho, dorsal derecho, dorsal izquierdo y subpneumostomal). Riñón sigmurétrico, sin uréter secundario. Dientes central y primeros laterales de la rádula provistos de ectoconos pequeños, pero nítidos. Pene diferenciado del epifalo por su anatomía interna y envuelto por una vaina penana. Músculo retractor inserto en el epifalo. Vagina tubular, sin trazas del aparato estimulador, con una o varias glándulas vaginales digitiformes, que tienen la base de menor diámetro que el resto y están dispuestas, cuando hay más de tres, formando una corona. El músculo retractor del ommatóforo derecho pasa entre el pene y la vagina. El nervio peneano se origina aparentemente del ganglio cerebroideo derecho.

Observaciones

Los caracteres del collar del manto y del complejo paleal son similares en todas las especies (con la excepción de *C. eutropis*), omitiéndose en ellas su descripción, así como la de otros caracteres comunes, para evitar repeticiones.

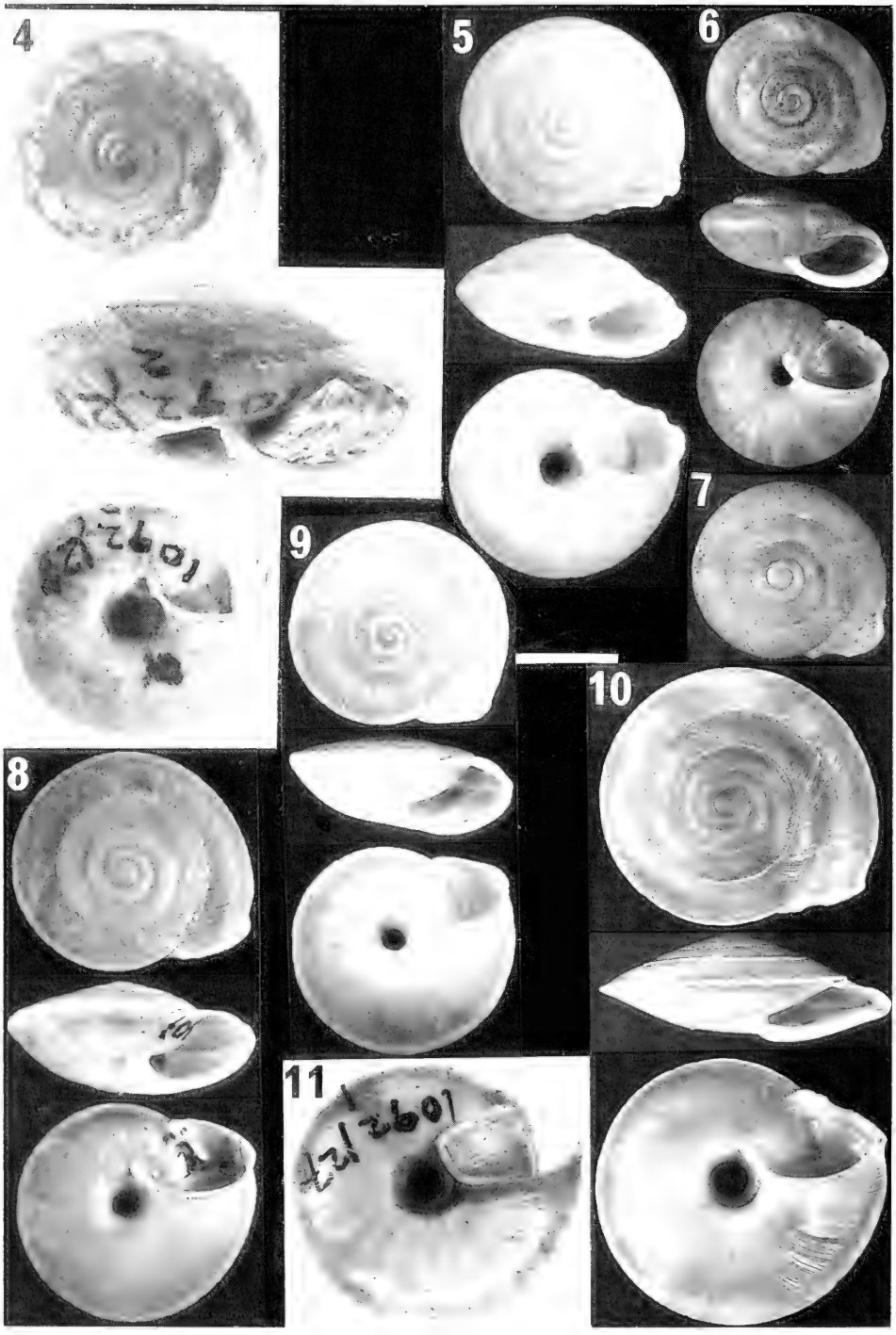
Canariella hispidula (Lamarck, 1822)

Helix (Helicigona) lens Férussac, 1821: 41 (Folio) o 37 (Quarto), no. 153 [*nomen nudum; non Helix lens,*— Deshayes, in Férussac & Deshayes, 1850 (= *Lindolmiola lens,*— Gittenberger & Groh, 1986)]; d'Orbigny, 1839: 66, lám. 2 figs. 7–9; Gray, 1854: 11; Chevallier, 1965: 489.

Carocolla hispidula Lamarck, 1822: 99 [loc. typ.: Tenerife]; 1838: 148; Mermod, 1951: 713–715, fig. 68 [1: lectotipo; 2: paralectotipo].

Helix (Helicigona) barbata Férussac, in Férussac & Deshayes, 1832: lám. "66*," fig. 4 [no. fig. 3; non *H. barbata* Férussac, 1821].

Helix hispidula,— Webb & Berthelot, 1833:



FIGS. 4–11. Concha. (4–9) *Canariella hispidula*. (4) Lectotipo de *Carocolla hispidula* (MHNG, foto G. Dajoz; diámetro de la concha: 13 mm). (5) Holotipo de *Helicodonta salteri* (NHM). (6) Lectotipo de *Helix bertheloti* (MNHN). (7) Lectotipo de *Helix everia* (MNHN). (8) Lectotipo de *Helix fortunata* (NMB). (9) Holotipo de *Helix (Gonostoma) beata* (NHM). (10–11) *Canariella planaria*. (10) Lectotipo de *Helix (Helicigona) afficta* (MNHN). (11) Lectotipo de *Carocolla planaria* (MHNG, foto G. Dajoz). Escala: (5–11) 5 mm.

TABLA 1. Datos biométricos (dimensiones en mm, e índices) de la concha en las diferentes variedades de *Canariella hispidula*. A: altura de la concha; B: diámetro de la concha; C: altura de la última vuelta; D: altura del lado ventral de la concha; E: longitud de la abertura; F: anchura de la abertura; G: diámetro del ombligo (sin peristoma); H: altura del lado dorsal de la concha (= A−D); n: número de ejemplares medidos. VALORES: M, valor máximo; m, valor mínimo; X, valor medio; CV: coeficiente de variación de Pearson (en %).

	A	B	C	D	E	F	G	A/B	H/B	D/B	E/F	B/G	n
C. hispidula var. *hispidula*													
M	7.11	15.33	5.66	4.65	6.26	7.19	3.36						
m	5.14	11.74	4.57	3.33	4.92	5.24	2.01						
X	6.31	13.15	5.27	4.01	5.69	6.26	2.51	0.48	0.17	0.31	0.91	5.31	24
CV	5.00	4.49	3.78	6.63	4.19	5.84	10.61	5.96	15.94	5.63	2.85	9.23	
var. *bertheloti*													
M	7.08	12.71	5.68	4.53	5.85	6.47	2.06						
m	5.45	9.41	4.52	3.08	4.34	4.50	0.96						
X	6.15	10.95	5.03	3.73	4.99	5.58	1.33	0.56	0.22	0.34	0.90	8.38	28
CV	5.84	6.46	5.60	7.71	6.39	7.76	12.33	3.69	13.75	5.52	5.20	10.82	
var. *fortunata*													
M	6.34	14.30	5.19	4.20	5.97	7.55	2.95						
m	5.40	12.07	4.57	3.42	4.93	5.74	1.83						
X	5.94	13.21	4.90	3.92	5.47	6.49	2.44	0.45	0.15	0.30	0.84	5.48	15
CV	3.83	4.02	3.01	3.52	5.09	4.20	8.37	3.45	12.52	5.16	3.55	7.92	
var. *beata*													
M	6.06	12.82	4.86	3.60	5.49	5.70	2.47						
m	4.71	10.07	3.92	2.87	4.28	4.73	1.33						
X	5.11	11.16	4.21	3.17	4.65	5.22	1.79	0.46	0.17	0.28	0.89	6.42	12
CV	4.96	5.49	4.80	5.53	5.32	4.92	15.46	5.92	13.01	4.62	5.14	12.47	
var. *subhispidula*													
M	7.07	11.99	5.62	4.18	5.35	6.43	2.19						
m	5.41	9.75	4.38	3.21	4.44	4.87	1.11						
X	6.32	11.09	5.13	3.80	4.95	5.62	1.65	0.57	0.23	0.34	0.88	6.87	17
CV	5.48	3.98	4.56	4.89	4.41	5.47	11.81	3.10	8.62	3.75	3.35	10.29	
var. *lanosa*													
M	5.40	8.98	4.44	3.44	4.18	4.66	0.39						
m	4.61	7.56	3.70	2.74	3.24	3.81	0.05						
X	4.96	8.22	4.04	3.09	3.78	4.30	0.21	0.61	0.23	0.38	0.88	53.73	13
CV	4.61	4.73	4.09	5.25	5.58	5.89	40.03	4.38	12.91	3.10	4.88	49.81	
Conjunto de todas las variedades													
M	7.11	15.33	5.68	4.65	6.26	7.55	3.36						
m	4.61	7.56	3.70	2.74	3.24	3.81	0.05						
X	5.93	11.47	4.87	3.69	5.02	5.67	1.71	0.52	0.20	0.33	0.89	12.26	109
CV	8.76	12.06	8.54	9.69	10.56	11.03	36.33	10.60	18.82	8.66	4.50	80.68	

314; Pfeiffer, 1848: 209; 1868: 260; 1876: 294–295 [*partim*]; Deshayes, in Férussac & Deshayes, 1851: 372–373; Mabille, 1884: 69–70; Krause, 1895: 25, fig. 5; Kraepelin, 1895: 9 [*partim* (loc. = Güímar)]; Shuttleworth, 1975: lám. 2, fig. 6.

Helix bertheloti Férussac, 1835: 90; d'Orbigny, 1839: 65–66, lám. 2, figs. 4–6; Pfeiffer, 1876: 295; Mabille, 1884: 81.

Helix fortunata Shuttleworth, 1852a: 141; 1975: lám. 2, fig. 4; Pfeiffer, 1853: 162; 1868: 260; 1876: 296; González Hidalgo, 1869: 37–38; Smith, 1884: 276; Mabille, 1884: 81–82 [*partim*]; Krause, 1895: 25.

Helix berthelotii,— Gray, 1854: 11.

Helix (Gonostoma) hispidula,— Albers, 1860: 92; Mousson, 1872: 62–63 [*partim*].

Helix (Ciliella) lanosa Mousson, 1872: 61–62, lám. 3, figs. 34–36; Pfeiffer, 1870–76: 83, lám. 122, figs. 34–36; 1876: 273–274; Mabille, 1884: 67; Tryon, 1887: 223, lám. 53, figs. 30–32.

Helix (Gonostoma) hispidula subhispidula Mousson, 1872: 63.

Helix (Gonostoma) bertheloti,— Mousson, 1872: 63–64.

Helix (Gonostoma) fortunata,— Mousson, 1872: 64 [*partim*]; Wollaston, 1878: 389–390 [*partim*].

Helix (Hispidella) lanosa,— Wollaston, 1878: 384–385.

Helix (Gonostoma) beata Wollaston, 1878: 390–391; Mabille, 1884: 85.
Helix everia Mabille, 1882: 147; 1884: 71, lám. 17, fig. 13.
Helix (Anchistoma) hispidula subhispidula,— Tryon, 1887: 122.
Helix (Anchistoma) everia,— Tryon, 1887: 123, lám. 38, figs. 4–6.
Helix (Anchistoma) fortunata,— Tyron, 1887: 123, lm. 24, figs. 55–57.
Helix (Caracollina) beata,— Tryon, 1887: 123.
Hygromia (Ciliella) lanosa,— Pilsbry, 1895: 276; Gude, 1896: 18.
Helicodonta (Caracollina) hispidula subhispidula,— Pilsbry, 1895: 288; Gude, 1896: 19.
Helicodonta (Caracollina) everia,— Pilsbry, 1895: 289.
Helicodonta (Caracollina) fortunata,— Pilsbry, 1895: 289; Gude, 1896: 19 [*partim*].
Helicodonta (Caracollina) beata,— Pilsbry, 1895: 289; Gude, 1896: 2: 19.
Helicodonta (Caracollina) hispidula everia,— Gude, 1896: 19.
Gonostoma hispidula,— Boettger, 1908: 246–247.
Gonostoma fortunata,— Boettger, 1908: 247.
Helicodonta salteri Gude, 1911: 268.
Canariella hispidula,— Hesse, 1918: 107; Odhner, 1931: 86, figs. 37C, 38C, 40A, 41A; Richardson, 1980: 422.
Canariella fortunata,— Hesse, 1931: 54–55, lám. 8, fig. 67a-e; Odhner, 1931: 87, figs. 37C, 38D; Richardson, 1980: 422; Gittenberger & Groh, 1986: 222–223.
?*Canariella leprosa,*— Hesse, 1931: 55, lám. 8, fig. 68a–b.
Canariella everia,— Odhner, 1931: 14: 87.
Canariella hispidula bertheloti,— Richardson, 1980: 422.
Caracollina beata,— Richardson, 1980: 423.
Caracollina everia,— Richardson, 1980: 424.

Es una especie polimorfa conquiológicamente, con seis poblaciones diferenciadas, siendo ésto un indicio de que se están iniciando diversos procesos de especiación, aunque no hay aislamiento geográfico real entre ellas y el aparato reproductor es muy similar en todas (para su estudio, se han disecado 64 ejemplares del total de las variedades: 13 de la forma típica; 17 de la var. *bertheloti;* 8 de la var. *fortunata;* 2 de la var. *beata;* 12 de la var. *subhispidula;* 12 de la var. *lanosa*). Por ello, consideramos que en la fase actual del proceso cada población no llega a alcanzar la categoría subespecífica y utilizamos sus nombres más antiguos para denominarlas, aunque dándoles el rango infrasubespecífico de variedad.

Como puede observarse en su mapa de distribución geográfica (Fig. 39), la var. *bertheloti* (representada en el mapa con estrellas) es la que ocupa una superficie mayor de la isla, en la vertiente Sur de las Cañadas del Teide y de la cordillera dorsal de La Esperanza, y también la que admite mayor variación altitudinal (se encuentra entre 100 y 1,625 m de altitud), lo que implica que se encuentra en una gran variedad de biotopos, con vegetación fundamentalmente de piso basal en las zonas bajas y de pinar en las altas, habiéndose recolectado además en jarales y en los escasos enclaves de bosque de laurisilva de esta vertiente de la isla. Su área de distribución se solapa ligeramente con la de la forma típica (var. *hispidula:* cuadrados blancos, que alcanza altitudes mucho menores, entre 10 y 490 m, con vegetación de piso basal) y entra en contacto con otras dos: la var. *subhispidula* (círculos) que se situa en las zonas altas cercanas a la dorsal de La Esperanza (en la vertiente sur, entre 1,000 y 1,700 m de altitud y con vegetación de pinar y también de fayal-brezal) y la var. *lanosa* (cruces), en la vertiente norte, que alcanza hasta la zona norte del macizo montañoso de Anaga (situado en el extremo Nordeste de la isla), entre 450 y 1,500 m de altitud, con vegetación de laurisilva, fayal-brezal y pinar; en la actualidad, su área de distribución está interrumpida entre la dorsal de La Esperanza y Anaga, debido a la destrucción por el hombre de su biotopo natural. Finalmente, al norte del área ocupada por la var. *hispidula,* en la vertiente sur del macizo de Anaga y con biotopos similares, se localizan la var. *fortunata* (cuadrados negros, entre 50 y 550 m de altitud) y la var. *beata* (triángulos, entre 80 y 350 m de altitud).

Material Examinado

Material tipo (conchas vacías, de Tenerife).—Lectotipo (MHNG 1092/28/2, col. Lamarck, selec.: E. Ponte-Lira y M. Ibáñez) y un paralectotipo (MHNG 1092/28/1) de *Carocolla hispidula.* Holotipo de *Helicodonta salteri* (NHM 1922.8.29.33). Lectotipo (selec.: K. Groh) y 2 paralectotipos de *Helix bertheloti* (MNHN, com. Webb, coll. Férussac). Lectotipo (selec.: K. Groh) y 5 paralectotipos de *Helix everia* (MNHN, 4, rec. Dr. Vernau y 1, rec. Bourgeau). Lectotipo (selec. E. Ponte-

Lira y M. Ibáñez) y 7 paralectotipos de *Helix fortunata* (NMB 307, Blauner, 1851), de Santa Cruz, y otros 2 (ZMZ 508672/partim, Blauner, 1852) de Santa Cruz. Holotipo de *Helix (Gonostoma) beata* (NHM 95.2.230, leg. Barón de Paiva), de "Betancuria, Fuerteventura" (localidad errónea). Lectotipo (selec.: E. Ponte-Lira y M. Ibáñez) y 9 paralectotipos de *Helix (Gonostoma) hispidula subhispidula* (ZMZ 508663, leg. Fritsch), de Paso Alto, y otros 6 (ZMZ 508665/5, leg. Fritsch, 1863 y 508664/1). Lectotipo (selec.: E. Ponte-Lira y M. Ibáñez) y 1 paralectotipo de *Helix (Ciliella) lanosa* (ZMZ 506132, Tarnier, 1865).

Otro material (todo, de Tenerife).—Var. *hispidula.*—5 conchas (ZMZ 508668/2, de Taganana y 508666/3, Fritsch, 1862); 1 (ANSP 248295/partim, P. Hesse, ex. Preston, 1912), de Santa Cruz; 15 (DMNH 15436/7, C. L. Richardson y 128654/8, R. Jackson), de Candelaria; 3 (MHNG 984/201); 2 (MNHN, Maugé); 5 (CGH) y 36 (TFMC), de Candelaria; 6 (TFMC, J. F. Guerra, 1953), de la calle Enrique Wolfson, Sta. Cruz. Var. *bertheloti.*—4 conchas (NHM 1854.9.28.39, Webb y Berthelot); 4 (FMNH 94096, C. D. Nelson), de La Orotava; 2 (FMNH 37784, G. K. Gude, ex. G. S. Parry); 5 (FMNH 37783, G. K. Gude, ex. H. B. Preston); 1 (ZMZ 508669, Tarnier, 1864), de Güímar; 3 (ZMZ 508660, Wollaston, 1860), de La Orotava y Santa Cruz; 2 (ZMZ 508661, Fritsch, 1863); 4 (ZMZ 508658, Blauner, 1852); 1 (ANSP 248296, Hesse, ex. Preston), de Güímar; 6 (DMNH 128692, R. Jackson), de Güímar; 5 (NMB, Blauner, 1851), de Güímar; 5 (MNHN, Jousseaume, Letellier, 1949 y Bourgeau, 1856); 7 (TFMC, J. M. Fernández, 1965), de la Ladera de Güímar. Var. *fortunata.*—6 conchas (FMNH 158.206), de Candelaria; 6 (FMNH 158.213), de La Resbalada; 6 (NMW, Melvill-Tomlin, 4 de ellos, de Santa Cruz); 2 (ZMZ 508671, Wollaston, 1870), de Santa Cruz; 4 (ZMZ 508667, Fritsch, 1872); 1 (ANSP 97264, Wollaston); 4 (ANSP 248295/partim, Hesse, ex. Preston), de Santa Cruz; 1 (ANSP 1563, A. D. Brown); 1 (ANSP 1514, A. D. Brown), de "Gran Canaria"; 2 (DMNH 151668, R. Jackson); 2 (MHNG, Moricand); 2 (MHNG); 2 (MNHN, Denis); 45 (TFMC, F. Guerra y J. M. Fernández), del Bco. Tahodio; 4 (TFMC), del Bco. de Santos; 4 (TFMC), de Las Mesas; 11 (TFMC), de la Ladera de Pino de Oro. Var. *beata.*—5 conchas (NHM 1854. 9.28.35, Webb y Berthelot), de Santa Cruz; 8 (MNHN/2, Locard, MNHN/2, M. Delaunay, 1882 y MNHN/4, Bourgeau, 1885); 6 (MNHN, Vernau, 1877–78), del bosque de Las Mercedes; 1 (MNHN, Jousseaume); 1 (ANSP 5111, A. D. Brown). Var. *subhispidula.*—6 conchas (FMNH 158.211), de El Palmar; 1 (ZMZ 508670); 2 (ANSP 33232, Wollaston); 4 de "*Helix bertheloti*" (NHM 1854.9.28.34, d'Orbigny). Var. *lanosa.*—1 concha (TFMC, J. M. Fernández, 1955), del Monte Las Mercedes; 23 (TFMC), de Agua García, S. Diego y Bco. Carnicería. Además, 750 conchas y 243 ejemplares en alcohol, de las seis variedades, recolectados entre los días 5-05-1979 y 15-01-1994, en diversas localidades de la isla (Fig. 39).

Forma típica: var. *hispidula* (Lamarck, 1822)
Helicodonta salteri Gude, 1911

Descripción (Tabla 1, Figs. 2–5, 28)

El animal tiene el cuerpo de color gris con manchas más oscuras, alargadas, que se disponen en filas longitudinales en el dorso de la cabeza. La concha es aplanado-lenticular, con 4 a 5½ vueltas de espira, la última angulada. La sutura es nítida, el ombligo, grande y la abertura redondeada. El color es marrón claro, sin brillo. Las costulaciones radiales son muy suaves en la protoconcha y están algo más desarrolladas en las siguientes vueltas de espira; en la última son irregulares, debido a que se deforman al rodear bases de pelos, que están presentes en gran número; por ello, en ocasiones se fusionan unas con otras o bien se interrumpen; en el lado ventral son más suaves que en el dorsal. Superpuesta a esta ornamentación hay otra, formada por crestas espirales muy finas y numerosas. La concha está densamente cubierta de pelos periostracales finos y largos, sobre y entre las costulaciones, que se desprenden fácilmente junto con el periostraco, quedando en su lugar protuberancias pequeñas. Su longitud varía mucho en cada vuelta de espira. En el lado dorsal son pequeños, menores de 100 µm, salvo en la zona de la sutura con la siguiente o en la periferia de la última, donde son mucho mayores, alcanzando hasta 800 µm. En el lado ventral son menores y su longitud disminuye hacia el ombligo, en el que no sobrepasan los 160 µm.

Mandíbula con más de 15 costillas. La rádula tiene la siguiente fórmula: (C + 17–25L) × 95–115. Los dientes cercanos al borde radular tienen el ectocono dividido en dos dentículos.

Aparato reproductor. La distancia del atrio

a la inserción del músculo retractor del pene es ligeramente mayor que la del resto del epifalo, que a su vez es más del doble de largo que el flagelo. Epifalo y flagelo son tubulares y adelgazan paulatinamente hasta el final del flagelo, que termina en forma de dedo de guante (no es puntiagudo). El pene tiene un pequeño estrangulamiento justo antes de desembocar en el atrio, y está ensanchado asimétricamente en su porción proximal, formando una pequeña protuberancia. El epifalo alberga cuatro pliegues longitudinales, que están bien desarrollados entre el flagelo y la inserción del músculo retractor, mientras que hacia la zona distal uno de ellos se va atenuando y termina desapareciendo antes de la unión con el pene. De los tres que quedan, uno termina en el extremo proximal del pene y los otros dos se prolongan en su cavidad y se fusionan formando una papila acanalada, situada en el mismo lado que la inserción del músculo retractor y cuya longitud equivale a casi la mitad del pene. A continuación de ella, el pene presenta 4–5 boceles cortos y gruesos, que lo recorren longitudinalmente hasta su inserción en el atrio. La vagina es tubular y en su interior posee 5 a 6 boceles longitudinales, que no conectan con los pliegues del conducto de la bolsa copulatriz. Un poco por debajo del orificio de comunicación con el oviducto, desembocan en ella un número variable de glándulas vaginales digitiformes (de 3 a 8), dispuestas formando un círculo a su alrededor.

El nervio peneano se origina del ganglio cerebroideo derecho.

var. *bertheloti* (Férussac, 1835) (Tabla 1, Figs. 6–7, 29). *Helix everia* Mabille, 1882

La concha es deprimida y algo más pequeña, con la espira menos angulada y el ombligo más pequeño. Las costulaciones radiales de la teloconcha están más desarrolladas y más próximas entre sí. El número de pelos periostracales es netamente inferior, variando su tamaño de 200 a 800 µm de longitud en el lado dorsal y no pasan de 110 µm en el ombligo. Con 3 a 6 glándulas vaginales.

var. *fortunata* (Shuttleworth, 1852) (Tabla 1, Figs. 8, 25, 30).

La concha es más aplanada, con la espira muy angulada y la abertura ovalada. Los pelos periostracales están dispuestos desordenadamente; los de la periferia alcanzan hasta 800 µm de longitud, mientras que en el ombligo no sobrepasan los 100 µm. Con 6 a 7 glándulas vaginales.

var. *beata* (Wollaston, 1878) (Tabla 1, Figs. 9, 31).

La concha es más aplanada y algo más pequeña, con la espira aquillada, la abertura ovalada y el ombligo más pequeño. Las costulaciones radiales son muy numerosas en la teloconcha. En los ejemplares mejor conservados, la pilosidad es muy escasa y se localiza en la periferia de la concha, siendo los pelos muy cortos (no pasan de 350 µm de longitud); los del ombligo son todavía más cortos, alcanzando hasta 100 µm. Los dos reproductores examinados únicamente poseen dos glándulas vaginales.

Observaciones

El holotipo (Fig. 9) de *Helix beata* (que es el único ejemplar sobre el que se basó Wollaston para describirla) tiene el lado dorsal de la concha muy aplanado, existiendo otros ejemplares con él más alto. Es probable que el error en el dato de la localidad típica ("Betancuria, Fuerteventura") se deba a un cambio de etiqueta o bien de la caja en que el ejemplar estuviera almacenado, desde que fue recolectado por el Barón de Paiva hasta que fue estudiado por Wollaston.

var. *subhispidula* (Mousson, 1872) (Tabla 1, Figs. 14, 32).

La concha es deprimida y algo más pequeña, con la espira ligeramente angulada, la abertura ovalada y el ombligo más pequeño. Las costulaciones radiales de la teloconcha están bien desarrolladas. Los ejemplares mejor conservados presentan una pilosidad muy escasa, situada en el lado dorsal de la última vuelta de espira y en la periferia de la concha, normalmente en los espacios intercostales. Los más largos son los de la periferia, que alcanzan hasta 700 µm de longitud; los del ombligo, en cambio, son muchísimo más cortos, no pasando de 60 µm. Con 4 a 6 glándulas vaginales.

var. *lanosa* (Férussac, 1835) (Tabla 1, Figs. 15, 26, 33).

La concha tiene forma cónico-ovalada y es bastante más pequeña, con la espira redondeada. El ombligo es muy pequeño, estando en algunos ejemplares casi totalmente cu-

bierto por el peristoma, y la abertura es ovalada. Toda la concha está cubierta de pelos periostracales finos y largos, que en el lado dorsal de cada vuelta son menores de 170 µm, salvo en la zona de la sutura con la siguiente o en la periferia de la última vuelta, en donde están los más grandes, que llegan a alcanzar cerca de 550 µm de longitud. En el interior del ombligo, no sobrepasan los 100 µm. Con 3 a 5 glándulas vaginales.

Observaciones

Es la población que más se diferencia conquiológicamente de las otras, evidenciando que su proceso de especiación es el más avanzado.

Canariella discobolus (Shuttleworth, 1852)

Helix discobolus Shuttleworth, 1852b: 290 [loc. typ.: Gomera]; 1975: lám. 6, fig. 6; Pfeiffer, 1853: 643; 1868: 260; 1870–76: 84, lám. 123, figs. 1–2; 1876: 296.
Helix afficta Férussac,—d'Orbigny, 1839: 66, lám. 3, figs. 24–26; Gray, 1854: 11 [*non H. afflicta* Férussac, 1821].
Helix (Gonostoma) discobolus,—Albers, 1860: 92; Mousson, 1872: 66, lám. 4, figs. 1–2.
Helix (Anchistoma) discobolus,—Tryon, 1887: 123, lám. 24, figs. 41–42.
Helicodonta (Caracollina) discobolus,—Pilsbry, 1895: 289; Gude, 1896: 19.
Canariella discobolus,—Hesse, 1931: 55–56, lám. 9, fig. 69a–b.
Canariella discobola,— Richardson, 1980: 422.

Material Examinado

1 concha (NHM 1854.9.28.46/partim, leg. Webb y Berthelot). Además, 64 conchas y 7 ejemplares en alcohol, recolectados en diversas localidades, entre los días 29-07-1982 y 30-01-1989.

Hábitat y Distribución (Fig. 43)

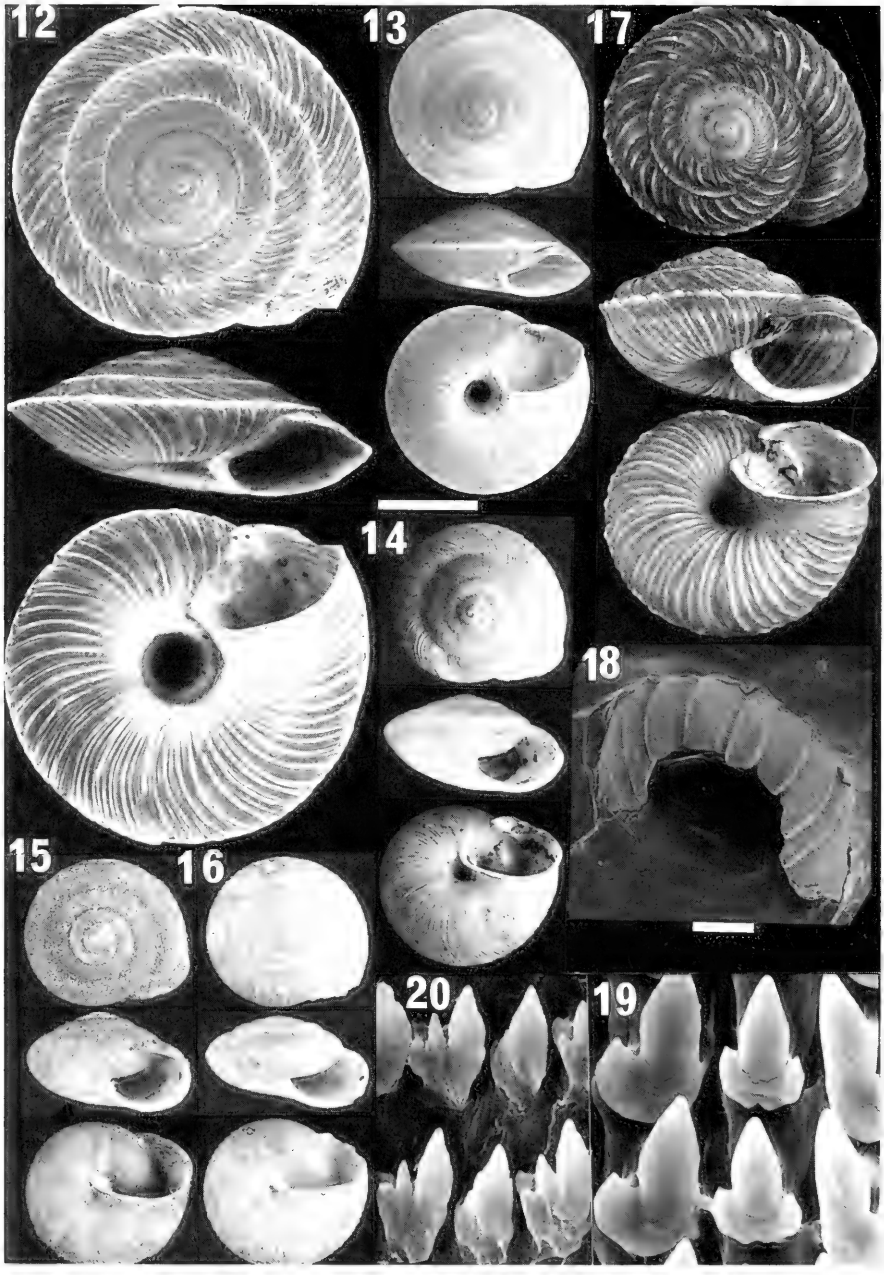
Endémica de La Gomera. Se distribuye en la zona sur de la isla, entre 175 y 800 m de altitud. Está ligada a zonas pedregosas con vegetación de piso basal.

Descripción (Tabla 2, Figs. 12, 34)

El animal fijado tiene el cuerpo de color blanquecino. La concha es aplanado-lenticular, con 6 a 6½ vueltas de espira, las primeras anguladas y las últimas aquilladas. La sutura es nítida, el ombligo grande y la abertura ovalada, angulada en la unión de las zonas palatal y basal. El color es marrón claro, sin brillo. Las costulaciones radiales son muy suaves en la protoconcha (donde se interrumpen dando la apariencia de una granulación) y en las primeras vueltas de espira, y están bien marcadas en las siguientes, siendo irregulares en la última. En el lado ventral son más regulares y más gruesas que en el dorsal, disminuyendo su grosor en las proximidades del ombligo y de la periferia. La pilosidad está restringida a la zona de la periferia, donde los pelos periostracales alcanzan hasta 500 µm de longitud, y al ombligo, donde son mucho más numerosos y cortos, no pasando de 45 µm.

Mandíbula con 10 a 13 costillas. La rádula tine la siguiente fórmula: (C + 18−30L) × 98−130. Los dientes cercanos al márgen radular tienen el ectocono dividido de forma irregular.

Aparato reproductor (se han disecado 3 ejemplares): la distancia del atrio a la inserción del músculo retractor del pene es igual que la del flagelo y menor que la del resto del epifalo. Las tres regiones son tubulares y van adelgazando paulatinamente desde el atrio hasta el final del flagelo. El epifalo alberga en su interior cuatro pliegues longitudinales, de los que dos terminan en su extremo distal y los otros dos se prolongan en la cavidad del pene; éstos, en dos de los reproductores examinados, engruesan ligeramente y terminan juntos, pero independientes entre sí, mientras que en el tercero se fusionan formando una pequeña papila acanalada, situada aproximadamente en el mismo lado que la inserción del músculo retractor. A continuación de ella, el pene presenta cuatro boceles (dos más gruesos) que lo recorren longitudinalmente hasta su inserción en el atrio. La vagina es gruesa y de tamaño similar al del pene; en ella desembocan dos glándulas vaginales digitiformes de tamaño variable y en su interior posee 6–7 boceles longitudinales delgados. Su pared presenta un engrosamiento muy desarrollado con aspecto almohadillado, que ocupa casi toda su cavidad y está dividido en cuatro lóbulos por un surco longitudinal y otro transversal. Entre los dos lóbulos proximales, cerca de su extremo, se encuentra el orificio de comunicación con el oviducto. El inicio del conducto de la bolsa copulatriz presenta internamente gran número de boceles con bordes irregulares.



FIGS. 12–20. Concha y SEM detalles. (12) *Canariella discobolus* (Barranco de la Rajita, La Gomera). (13) *Canariella gomerae.* Lectotipo de *Helix (Gonostoma) gomerae* (NHM; es un ejemplar pequeño dentro de la especie). (14–15) *Canariella hispidula.* (14) Lectotipo de *Helix (Gonostoma) hispidula subhispidula* (ZMZ). (15) Lectotipo de *Helix (Ciliella) lanosa* (ZMZ). (16) *Canariella leprosa* (El Draguillo, Tenerife). (17–18) *Canariella eutropis.* (17) Lectotipo de *Helix eutropis* (NMB). (18) Mandíbula de un ejemplar de Morro del Cavadero, Fuerteventura). (19–20) Rádula de *Canariella planaria* (Benijo, Tenerife). (19) Diente central y primeros dientes laterales. (20) Dientes laterales próximos al margen radular. Escala: (12–17) 5 mm; (18) 200 μm; (19–20) 20 μm.

TABLA 2. Datos biométricos (dimensiones en mm, e índices) de la concha de las especies de *Canariella*. Símbolos, como en la Tabla 1.

	A	B	C	D	E	F	G	A/B	H/B	D/B	E/F	B/G	n
Canariella discobolus													
M	7.81	20.21	5.97	5.56	7.11	9.73	4.94						
m	6.62	16.08	5.14	4.21	5.67	3.56	2.95						
X	7.16	18.14	5.61	5.03	6.41	7.93	3.95	0.40	0.12	0.28	0.85	4.65	17
CV	4.68	4.78	3.45	7.03	5.43	9.52	9.95	5.65	26.42	6.36	14.52	7.88	
Canariella gomerae													
M	6.55	13.79	5.16	4.53	5.69	6.67	2.73						
m	5.22	11.51	4.33	2.93	4.59	5.47	2.07						
X	5.89	12.54	4.74	3.80	5.01	5.91	2.36	0.47	0.17	0.30	0.85	5.35	11
CV	4.07	4.29	5.28	7.30	5.83	4.58	9.36	2.24	16.21	7.96	2.30	8.93	
Canariella planaria													
M	5.79	14.95	4.53	4.37	6.23	7.05	3.29						
m	4.11	12.70	3.24	2.76	4.53	5.69	2.17						
X	5.13	13.96	4.03	3.44	5.48	6.31	2.78	0.37	0.12	0.25	0.87	5.07	32
CV	5.07	2.98	5.50	8.84	4.96	4.74	8.29	4.52	18.17	8.31	4.78	8.94	
Canariella leprosa													
M	4.68	9.18	3.94	3.48	3.91	5.00	0.30						
m	4.25	7.97	3.49	2.71	3.50	3.90	0.20						
X	4.56	8.58	3.70	3.07	3.80	4.59	0.25	0.53	0.17	0.36	0.83	35.88	4
CV	3.35	5.16	5.60	9.19	3.95	7.54	20.00	2.49	15.89	4.05	4.09	21.81	
Canariella eutropis													
M	8.84	16.52	7.47	6.47	7.27	8.06	3.00						
m	7.32	13.82	5.88	4.83	5.08	6.47	1.62						
X	8.00	15.00	6.74	5.65	6.43	6.99	2.22	0.53	0.16	0.38	0.92	6.89	29
CV	4.33	2.81	4.36	5.70	3.95	4.06	12.03	4.26	16.96	4.68	4.80	11.29	

Canariella gomerae (Wollaston, 1878)

Helix (Gonostoma) gomerae Wollaston, 1878: 392–393 [loc. typ.: Hermigua, La Gomera]; Mabille, 1884: 84–85.

Helicodonta (Caracollina) gomerae,—Pilsbry, 1895: 289; Gude, 1896: 19.

Caracollina gomerae,—Richardson, 1980: 424.

Material Examinado

Material tipo (conchas vacío).—Lectotipo (selec.: E. Ponte-Lira y M. Ibáñez) y 3 paralectotipos de *Helix (Gonostoma) gomerae* (NHM 95.2.216–19), de Hermigua (La Gomera); y otro paralectotipo (FMNH 37781, colección de Gerard K. Gude, ex G. S. Parry; juvenil) de Hermigua. Otro material.—1 juvenil (NMW; Melvill-Tomlin collection). Además, 84 conchas y 19 ejemplares en alcohol, recolectados entre los días 3-01-1978 y 2-01-1993, en diversas localidades de la isla.

Hábitat y Distribución (Fig. 43)

Endémica de La Gomera. Se distribuye por la zona norte de la isla, entre 100 y 1,200 m de altitud, en varios tipos de vegetación, desde tabaibales a fayal-brezal y laurisilva.

Descripción (Tabla 2, Figs. 13, 21, 35)

El animal fijado tiene el cuerpo de color blanquecino. La concha es aplanado-lenticular, con 5½ a 6 vueltas de espira, las primeras anguladas y las últimas aquilladas. La sutura es nítida, el ombligo grande y la abertura ovalada, angulada en la unión de las zonas palatal y basal. El color es marrón claro, sin brillo. Las superficies dorsal y ventral están provistas de costulaciones radiales suaves y uniformes. Superpuesta a esta ornamentación hay otra, formada por crestas espirales muy finas y numerosas. Además, sobre las costulaciones y los espacios que hay entre ellas existen, en la última vuelta, pequeños gránulos redondeados, que son más patentes en la superficie ventral y posiblemente corrsponden a la base de los pelos periostracales. La pilosidad está restringida a la zona de la periferia, donde son finos y muy poco abundantes en los ejemplares adultos, alcanzando hasta 400–450 µm de longitud, y al ombligo, donde son mucho más numerosos y cortos (menores de 35 µm).

Mandíbula con más de 13 costillas. La rádula tiene la siguiente fórmula radular: (C + 25 − 31L) × 97 − 125. Los dientes cercanos

al margen radular tienen el ectocono a veces dividido en dentículos.

Aparato reproductor (se han disecado 3 ejemplares): la distancia del atrio a la inserción del músculo retractor del pene es ligeramente inferior a la del resto del epifalo y mayor que el flagelo. Éste, es largo y muy esbelto, con un diámetro ligeramente superior al del conducto deferente. El pene es casi cilíndrico y está engrosado en su extremo distal, disminuyendo paulatinamente su diámetro en sentido proximal. El epifalo alberga en su interior a cuatro pliegues longitudinales, de los que dos terminan en el extremo proximal del pene y los otros dos se prolongan en su cavidad, fusionándose en una papila acanalada pequeña (menor que 1/3 de la longitud del pene), situada en el mismo lado que la inserción del músculo retractor. A continuación de ella, el pene presenta cuatro boceles alargados que lo recorren longitudinalmente hasta su inserción en el atrio. La vagina es gruesa y más corta que el pene y en su porción proximal desembocan, a la misma altura, tres glándulas vaginales digitiformes; dos de ellas son de igual tamaño y la tercera es más corta. Internamente, la vagina posee 3–4 boceles longitudinales. En la parte proximal, justo debajo del orificio de comunicación con el oviducto, presenta un engrosamiento almohadillado de su pared, dividido en dos lóbulos por un surco longitudinal. El inicio del conducto de la bolsa copulatriz tiene en su interior gran número de boceles con bordes irregulares.

Canariella planaria (Lamarck, 1822)

Carocolla planaria Lamarck, 1822: 99 [loc. typ.: Tenerife, hic. restr.: Vertiente Norte de Tenerife, entre la Punta de Juan Blas y la Punta de Anaga]; 1838: 148; Mermod, 1951: 712–713, fig. 67 [paralectotipo].

Helix afficta Férussac, 1821: 41 (Folio) o 37 (Quarto), n° 151 [*nomen nudum;* ICZN, Art. 12]; Férussac, in Férussac & Deshayes, 1832 (Atlas), lám. 66*, fig. 5; Pfeiffer, 1848: 211; 1853: 162; 1868: 260; Deshayes, in Férussac & Deshayes, 1851: 372; Tryon, 1887: 122, lám. 24, figs. 52–54 [*partim*]; Chevallier, 1965: 489.

Helix afficta planaria,—Mousson, 1872: 65–66; Pfeiffer, 1876: 296.

Helix planaria,—Wollaston, 1878: 391–392; Mabille, 1884: 82–84; Tryon, 1887: 122, lám. 24 figs. 58–60 [*partim*].

Helicodonta planaria,—Gude, 1896: 19.

Caracollina planaria,—Richardson, 1980: 425.

Material Examinado

Material tipo (conchas vacías, de Tenerife).—Lectotipo (MHNG 1092/27/1, col. Lamarck, selec.: E. PonteLira y M. Ibáñez) y un paralectotipo (MHNG 1092/27/2) de *Carocolla planaria.* Lectotipo (selec.: K. Groh) y 3 paralectotipos de *Helix (Helicigona) afficta* Férussac, 1832 (MNHN; col. Férussac). Otro material.—3 conchas (MNHN); 4 (NMW); 4 (ZMZ 508677/2 y 508676/2) de Taganana; 1 (ZMZ 508678); 3 (FMNH 37785) y otras 2 (FMNH 37782) de Almáciga; 2 (DMNH 78297) de Guayonga; 7 (MHNG); 1 (NHM 1854.9.28.46/partim); 1 (ANSP 397001), de Montaña Tafada; 2 (ANSP A17996 y 397002) de Benijo. Además, 283 conchas y 37 ejemplares en alcohol, recolectados entre los días 3-03-1981 y 8-11-1993, en diversas localidades.

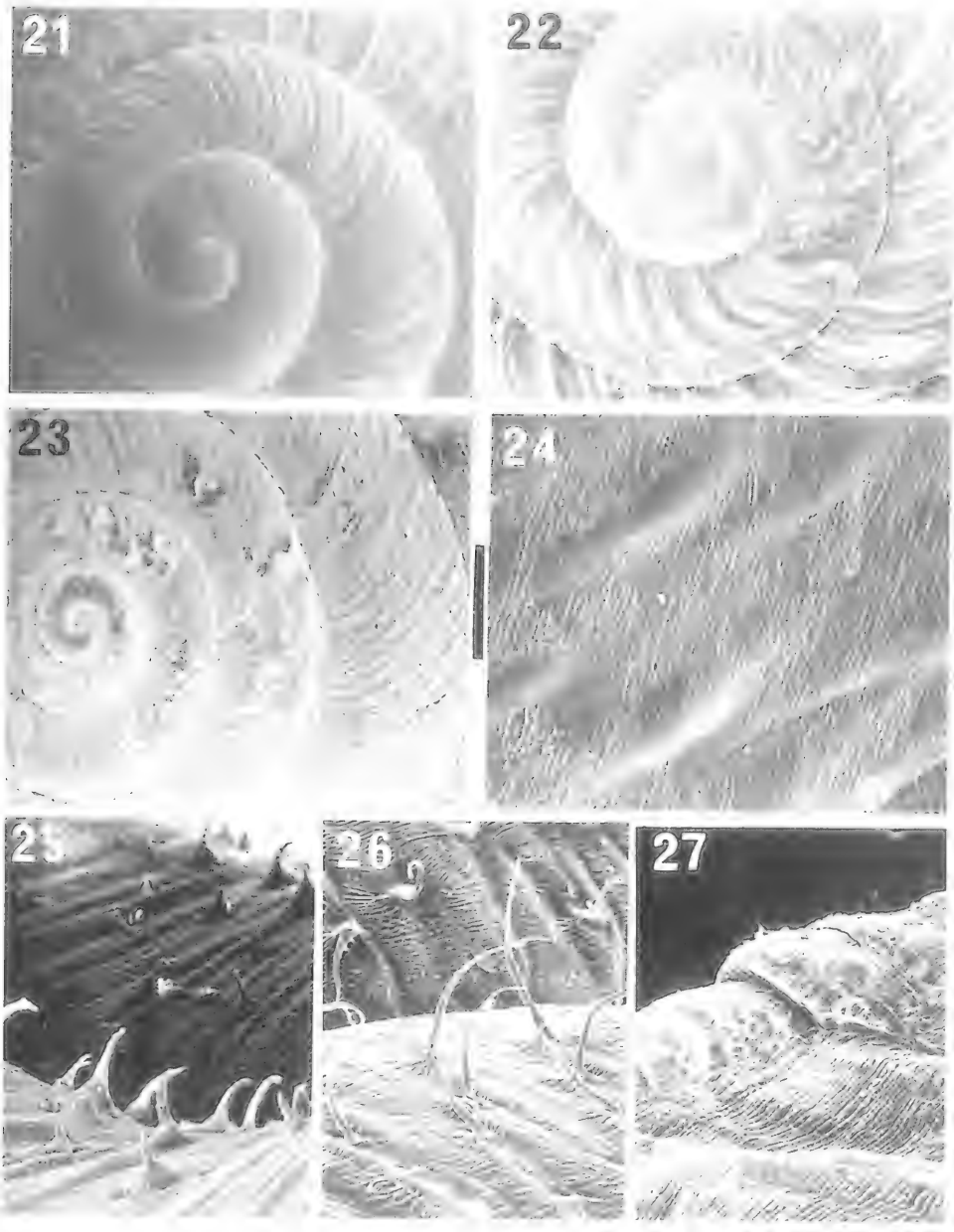
Hábitat y Distribución (Fig. 40)

Endémica de Tenerife. Habita en la vertiente norte de la isla, desde Punta de Juan Blas a Punta de Anaga, entre 5 y 800 m de altitud. Vive en zonas con vegetación muy diversa, normalmente en tabaibales.

Descripción (Tabla 2, Figs. 10, 11, 19, 20, 36)

El cuerpo es de color gris claro con manchas más oscuras, alargadas, dispuestas en filas longitudinales en el dorso de la cabeza. La concha es aplanado-lenticular, con 5 a 5½ vueltas de espira, las primeras anguladas y las últimas aquilladas. La sutura es nítida, el ombligo grande y la abertura ovalada, angulada en la unión de las zonas palatal y basal. El color es marrón claro, con un ligero brillo. Las costulaciones radiales son muy suaves en las primeras vueltas de espira y ligeramente más marcadas en las siguientes; en el lado ventral son más suaves que en el dorsal. Las crestas espirales en el lado ventral son finísimas y muy apretadas mientras que en el dorsal hay en su lugar algunas estrías espirales profundas, poco numerosas y espaciadas entre sí. La pilosidad está restringida a la zona del ombligo, donde los pelos son muy numerosos y cortos.

Mandíbula con más de 20 costillas. La rá-



FIGS. 21–27. SEM detalles de la concha. (21–23) Protoconcha y primeras vueltas de espira. (21) *Canariella gomerae* (Aguajilva, La Gomera). (22) *Canariella eutropis* (Morro del Cavadero, Fuerteventura). (23) *Canariella leprosa* (El Draguillo, Tenerife). (24) *C. leprosa.* Detalle de la última vuelta, lado dorsal. (25) *C. hispidula* var. *fortunata* (Cabezo de las Mesas, Tenerife). Detalle del ombligo. (26) *Canariella hispidula* var. *lanosa* (Agua García, Tenerife). Detalle de la penúltima y última vueltas. (27) *C. eutropis* (Morro del Cavadero). Quilla y ornamentación (última vuelta, lado dorsal). Escala: (21, 27) 500 μm; (22, 23) 1 mm; (24, 25) 125 μm; (26) 250 μm.

dula tiene la siguiente fórmula: (C + 27 – 31L) × 100 – 130. Los dientes cercanos al márgen radular poseen el ectocono dividido generalmente en dos dentículos de diferente tamaño.

Aparato reproductor (se han disecado 10 ejemplares): la distancia del atrio a la inserción del músculo retractor del pene es 2½ veces mayor que la del resto del epifalo. El flagelo es muy delgado y muy corto, casi rudimentario. El epifalo alberga en su interior cinco pliegues longitudinales (de los que dos son muy pequeños) que normalmente finalizan en su extremo distal; en un ejemplar, estos pliegues conectan con los boceles del pene, aunque estrechándose en la zona de contacto. En el interior del pene hay 7–8 boceles longitudinales, algunos de ellos anastomosados. El que está en el mismo lado que la inserción del músculo retractor, tiene en su extremo proximal un engrosamiento papiliforme muy nítido (no existe la papila peneana típica de las otras especies), y el situado en el lado opuesto engruesa considerablemente en posición distal. La vagina alberga en su interior un número variable de boceles longitudinales, algunos anastomosados, que se prolongan sin interrupción (estrechándose) en el conducto de la bolsa copulatriz. En su zona media desemboca una glándula vaginal digitiforme pequeña (como excepción, en un ejemplar encontramos dos glándulas).

Canariella leprosa (Shuttleworth, 1852)

Helix leprosa Shuttleworth, 1852a: 142 [loc. typ.: Tenerife, hic restr.: zona norte de Anaga]; *non Canariella leprosa,*—Hesse, 1931: 55, lám. 8, fig. 68a–b]; 1975: pl. 3, fig. 10; Pfeiffer, 1853: 130; 1870–76: 82–83, lám. 122, figs. 31–33; 1876: 273; ? Mabille, 1884: 66–67.

Helix (Ciliella) leprosa,—Mousson, 1872: 61, lám. 3, figs. 31–33; Tryon, 1887: 223, lám. 53, figs. 33–35.

Hygromia (Ciliella) leprosa,—Pilsbry, 1895: 276; Gude, 1896: 18.

Helix (Hispidella) leprosa,— Wollaston, 1878: 383–384.

Material Examinado

Una concha (ZMZ 506131; rec. Tarnier, 1865) de "Tenerife." Además, 24 conchas y 17 ejemplares en alcohol, recolectados entre los días 3-03-1981 y 15-01-1993, en diversas localidades.

Hábitat y Distribución (Fig. 41)

Endémica de Tenerife. Se distribuye por la zona norte de la isla, entre 400 y 800 m de altitud. Está ligada generalmente a bosques de laurisilva y fayal-brezal, habiéndose recolectado también en piso basal.

Descripción (Tabla 2, Figs. 16, 23, 24, 37)

El animal fijado tiene el cuerpo de color blanquecino. La concha es deprimida, con 4 3/4 a 5 vueltas de espira. La sutura es nítida, la abertura ovalada y el ombligo muy pequeño y casi completamente tapado por el peristoma. El color es marrón, sin brillo, pudiéndose desprender el periostraco parcialmente, incluso en los animales vivos. Las costulaciones radiales son pequeñas, no equidistantes, muy suaves (prácticamente inexistentes) en la protoconcha y en las primeras vueltas de espira y bien marcadas en las siguientes. A partir de la tercera vuelta, tienen una serie de interrupciones, mucho más patentes en la última, donde son sustituidas por filas de gránulos alargados que dan a la concha un aspecto muy característico, más acusado en el lado ventral. En las proximidades del ombligo, los gránulos están atenuados y desaparecen casi por completo. Superpuesta a esta ornamentación hay otra, formada por crestas espirales muy finas y numerosas, que se han desgastado sobre los gránulos dorsales. La pilosidad está restringida a la zona de la periferia, donde son finos y escasos, alcanzando hasta 360 μm de longitud.

Aparato reproductor (se han disecado 5 ejemplares): El atrio es corto; la distancia desde él hasta la inserción del músculo retractor del pene es alrededor del triple que la del resto del epifalo. El flagelo es muy delgado y muy corto, casi rudimentario. El epifalo alberga en su interior tres pliegues longitudinales, que en su extremo distal se fusionan en un grueso bocel del pene, que alcanza más de las 3/4 partes de la longitud del pene. La base de este bocel está unida a todo el perímetro interno del pene, realizándose la comunicación entre el epifalo y el pene a través de un pequeño conducto que atraviesa la base del bocel y se abre inmediatamente después, en su zona proximal. El resto de la pared del pene está ornamentado

con otros seis boceles, mucho más delgados. La vagina presenta en su pared interna cuatro boceles longitudinales finos, que no conectan con los pliegues (más finos y numerosos) del conducto de la bolsa copulatriz. Una única glándula vaginal digitiforme, grande y relativamente gruesa, desemboca cerca del extremo distal de la vagina.

Observaciones

Conquiológicamente es similar a *C. pthonera* por el tamaño y la ornamentación de la concha, pero ambas se diferencian claramente por el ombligo y la forma del la abertura (*C. pthonera* tiene ombligo grande y abertura redondeada). Con respecto a la anatomía del aparato reproductor, se diferencia claramente de las demás especies del género por la sustitución de la papila del pene por un grueso bocel perforado en su base.

Mousson (1872) creó el género *Ciliella* para agrupar, junto a *Ciliella ciliata* (Studer), dos especies de Canarias: *Helix leprosa* y *Helix lanosa*. Basándose probablemente en Mousson, varios autores (entre ellos, Germain, 1930; Thiele, 1931; Zilch, 1960; y Schileyko, 1991) indican que *Ciliella* se encuentra en Europa y en Canarias. Pero *Ciliella* carece de glándulas vaginales, que están presentes en las dos especies de *Canariella* (*H. leprosa y H. lanosa*). Podemos afirmar, por tanto, que *Ciliella* no tiene representates en el Archipiélago.

Canariella eutropis (Shuttleworth, 1860)

Helix eutropis Shuttleworth, in Pfeiffer, 1860: 237 [loc. typ.: montes de Jandía, Fuerteventura (figura en la etiqueta del material tipo)]; Albers, 1860: 139; Pfeiffer, 1868: 371; 1870–76: 81, lám. 122, figs. 28–30; Mousson, 1872: 58–59, lám. 3, figs. 28–30; Wollaston, 1878: 380; Mabille, 1884: 89–90; Tryon, 1888: 36, lám. 7, figs. 16–18; Pilsbry, 1895: 289; Gude, 1896: 19.

Material Examinado

Material tipo (conchas vacías).—Lectotipo (selec.: E. Ponte-Lira y M. Ibáñez) y 1 paralectotipo de *Helix eutropis* (NMB 139, "Dr. Bolle detexit"), de los montes de Jandía. Otro material.—2 conchas (FMNH 37788); 3 (ANSP 397000/2 y A17995) del Morro del Cavadero (Fuerteventura). Además, 54 conchas y 114 ejemplares en alcohol, recolectados entre los días 18-08-1986 y 8-03-1990, en diversas localidades de los montes de Jandía.

Hábitat y Distribución (Fig. 44)

Endémica de Fuerteventura. Habita en las zonas más húmedas de los montes de Jandía, entre 250 y 800 m de altitud.

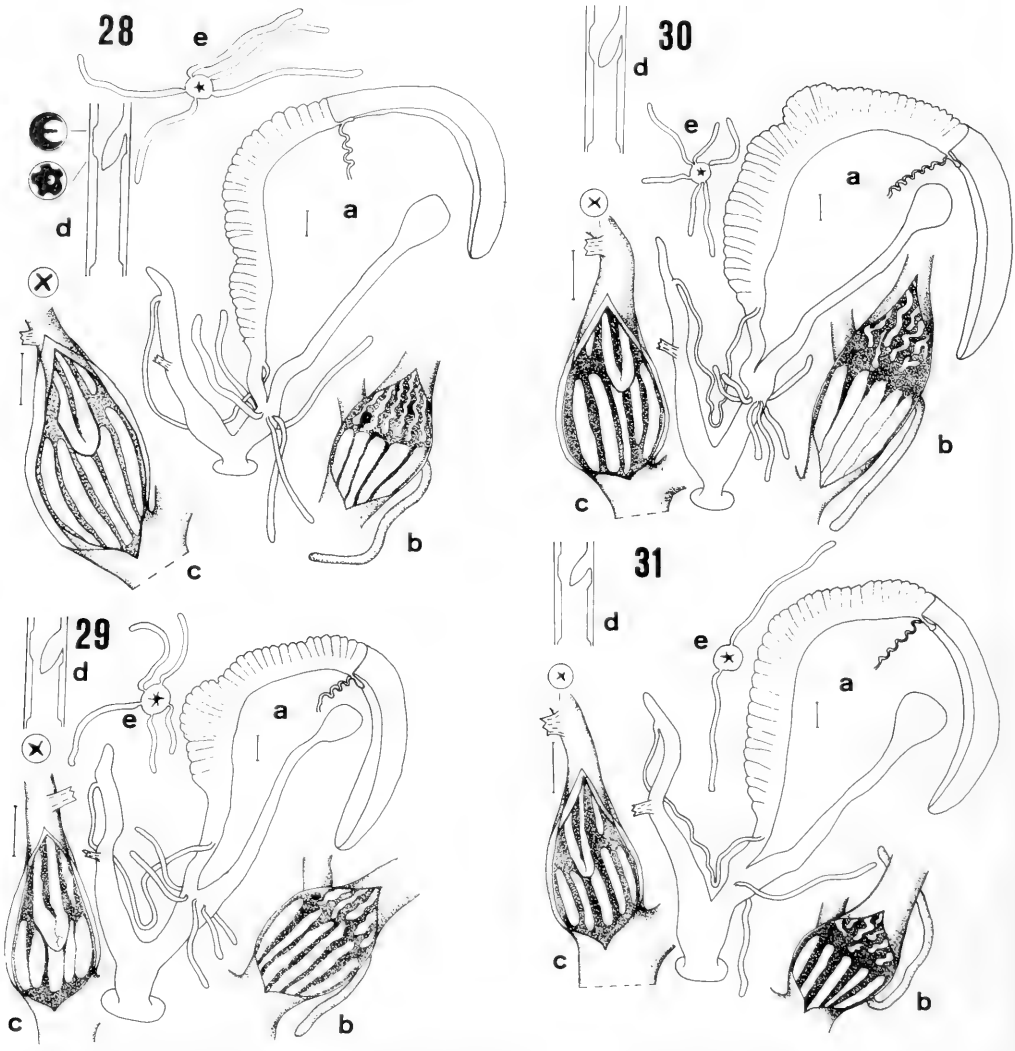
Descripcíon (Tabla 2, Figs. 17, 18, 22, 27, 38)

El cuerpo es de color gris con manchas más oscuras, alargadas, que se disponen en filas longitudinales en el dorso de la cabeza. La concha carece de pilosidad; es deprimida, aplanada en el lado dorsal y ovalada en el ventral, con 4½ a 5¼ vueltas de espira; a partir de la segunda, está provista de una quilla muy patente. La sutura es nítida, el ombligo grande y la abertura redondeada, aunque angulada en la unión de las zonas palatal y basal. El color es marrón amarillento, sin brillo, existiendo normalmente tres bandas espirales ligeramente más oscuras, dos dorsales y una ventral. Las costulaciones radiales son muy suaves en las primeras vueltas de espira, transformándose en costillas muy marcadas y distanciadas entre sí en las siguientes. En el lado ventral son más numerosas (alrededor de 45–50, frente a 35–37 del lado dorsal de la última vuelta). Algunas costillas del lado dorsal y todas las del ventral se prolongan sobre la quilla, que además también tiene otras protuberancias propias. Las crestas espirales son muy finas y numerosas, estando desgastadas sobre la quilla.

El collar del manto difiere de las demás especies descritas del género por la presencia del lóbulo lateral izquierdo, muy fino y poco conspicuo, que está situado en posición opuesta al pneumostoma.

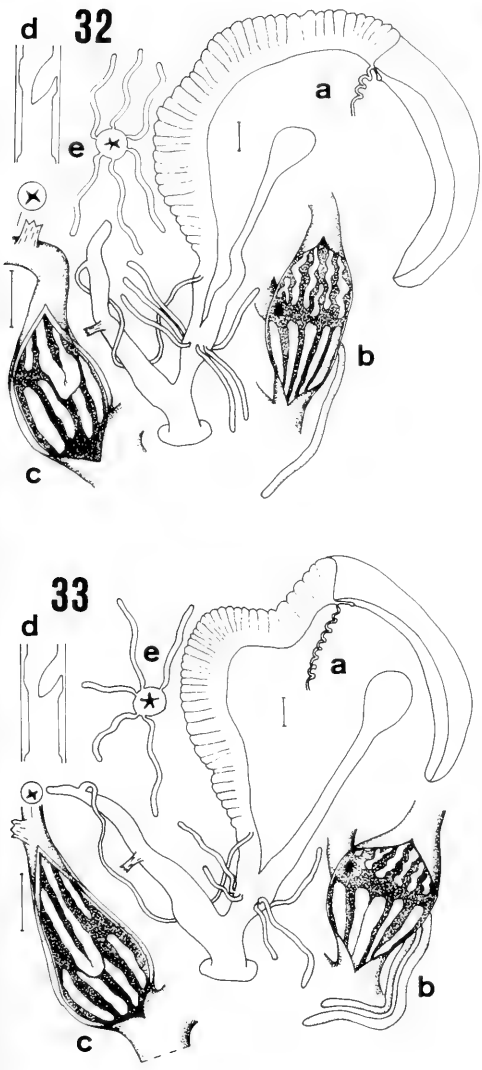
Mandíbula con 3 a 9 costillas. La rádula tiene la siguiente fórmula: (C + 28–32L) × 130–140. Los dientes cercanos al borde radular tienen el ectocono dividido en un número variable de dentículos, que le dan un aspecto aserrado.

Aparato reproductor (se han disecado 4 ejemplares): la distancia del atrio a la inserción del músculo retractor del pene es similar a la del resto del epifalo y doble a triple que la del flagelo. El epifalo alberga en su interior cinco pliegues longitudinales delgados, lisos

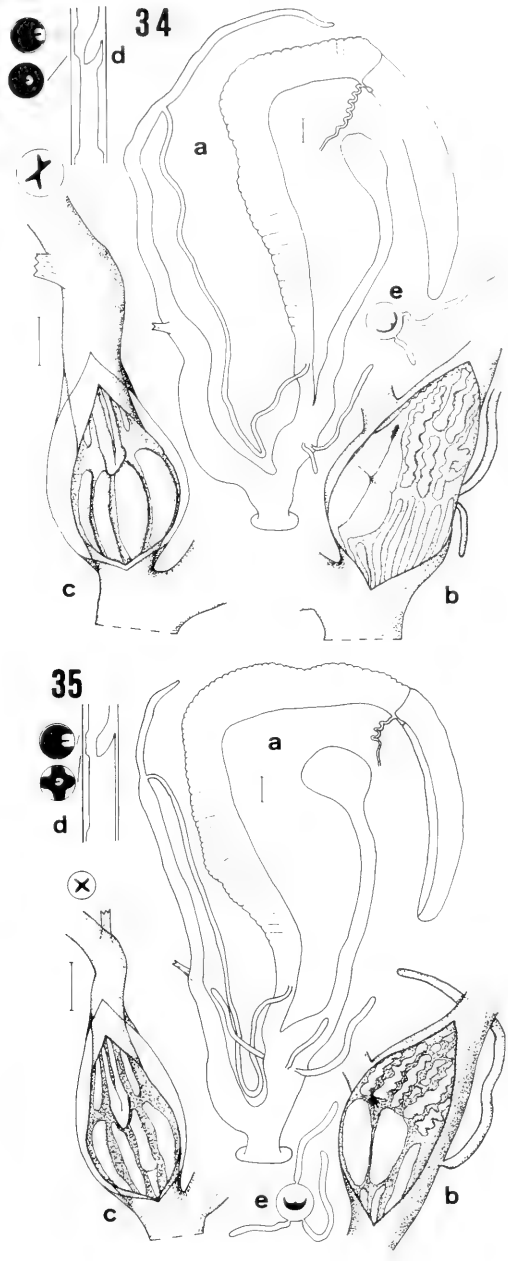


FIGS. 28–38. Aparato reproductor y detalles (escala: 1 mm); a: visión general; b: anatomía interna de la vagina y zona distal del conducto de la bolsa copulatriz; c: anatomía interna del pene y zona distal del epifalo; d: sección longitudinal del pene y transversales del pene y del epifalo (sin escala); e: disposición de las glándulas vaginales alrededor de la vagina; f: pene evaginado, mostrando la papila acanalada (*) (28) *Canariella hispidula* var. *hispidula* (Tabaiba Alta, Tenerife). (29) *C. hispidula* var. *bertheloti* (Arafo, Tenerife). (30) *C. hispidula* var. *fortunata* (Cabezo de las Mesas, Tenerife). (31) *C. hispidula* var. *beata* (Lomo Bermejo, Tenerife). (32) *C. hispidula* var. *subhispidula* (Montaña del Cascajo, Tenerife). (33) *C. hispidula* var. *lanosa* (Agua García, Tenerife). (34) *Canariella discobolus* (Barranco de La Rajita, La Gomera). (35) *Canariella gomerae* (Aguajilva, La Gomera). (36) *Canariella planaria* (Playa Fabián, Tenerife). (37) *Canariella leprosa* (Monte Tenejías, Tenerife). (38) *Canariella eutropis* (Morro del Cavadero, Fuerteventura).

en su porción proximal y ondulados desde la zona de la inserción del músculo retractor hasta el pene. Todos ellos se fusionan entre sí en su extremo distal, prolongándose en una papila peneana que en su base está unida a todo el perímetro interno del pene, cerrando completamente la luz del conducto (salvo su propio conducto interno), y en su porción libre es acanalada. Esta papila ocupa aproximadamente la mitad de la longitud del

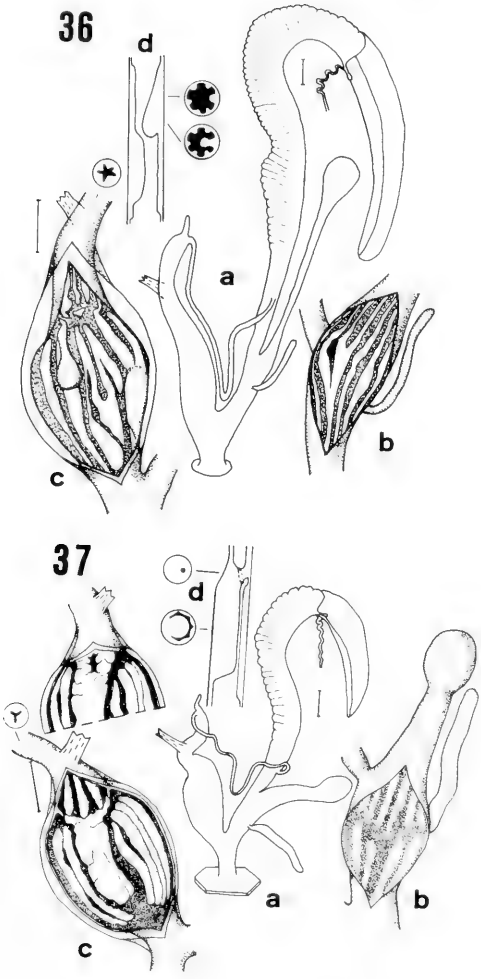


FIGS. 32 y 33.



FIGS. 34 y 35.

pene y de su base nace un grueso bocel longitudinal, situado en el mismo lado que la inserción del músculo retractor. Este bocel llega casi hasta el extremo distal del pene y su superficie está plegada transversalmente. Al evaginarse el pene, el bocel forma una protuberancia longitudinal, que se dispone inmediatamente detrás de la papila y aumenta considerablemente el grosor del pene evaginado; probablemente su función es evitar que éste se separe accidentalmente del otro individuo durante la cópula. Finalmente, el resto de la pared del pene está ornamentado con pequeños pliegues irregulares, generalmente transversales y a veces anastomosados entre sí. La vagina está recorrida en su interior por cuatro boceles longitudinales finos; cerca de su extremo proximal desembocan en ella 2–3 glándulas vaginales digitiformes pequeñas y relativamente gruesas. El



FIGS. 36 y 37.

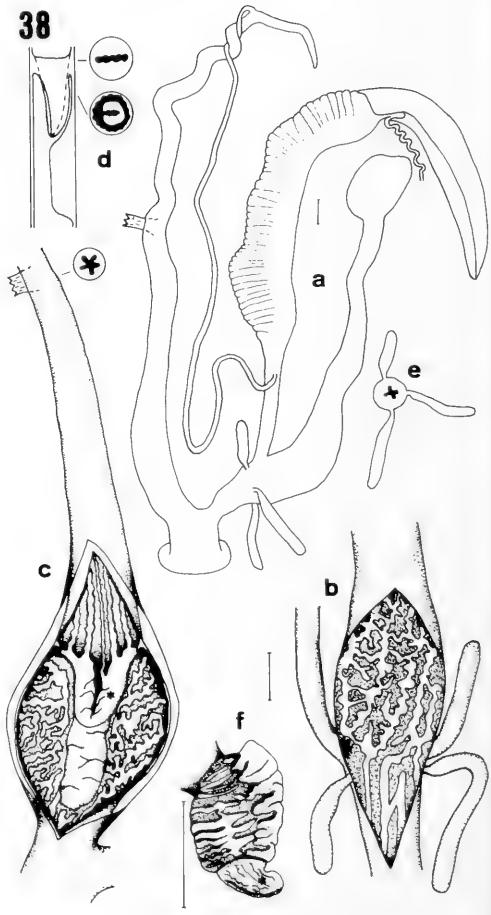


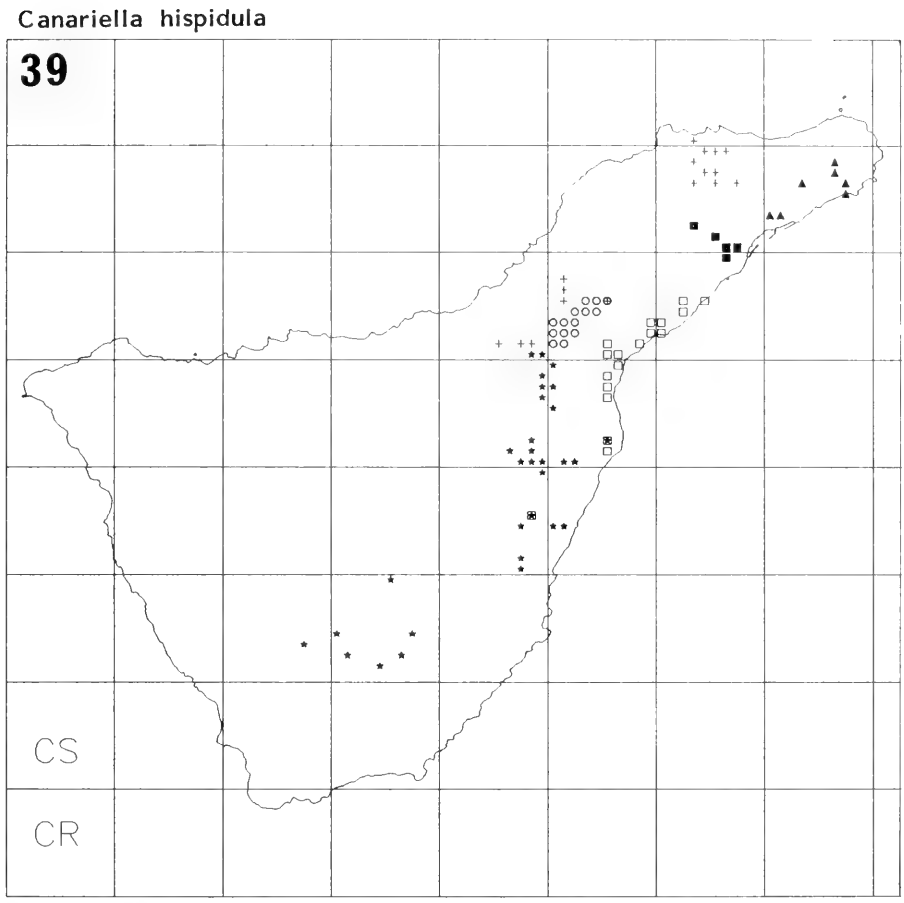
FIG. 38.

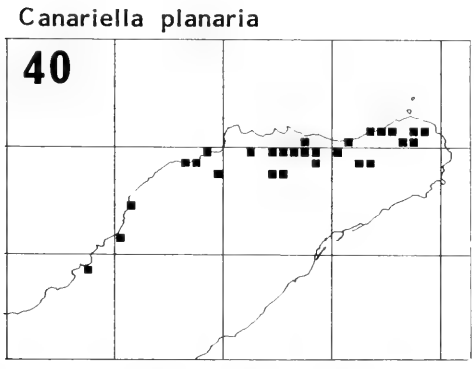
inicio del conducto de la bolsa copulatriz está provisto internamente de gran número de pliegues irregulares, anastomosados entre sí.

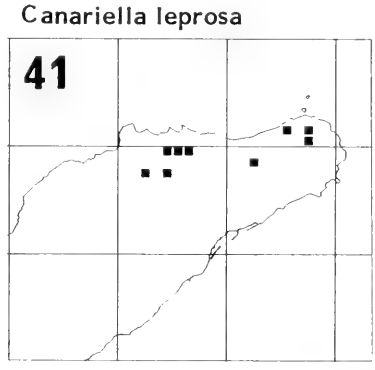
DISCUSIÓN

El género *Canariella* fue situado inicialmente por Hesse (1918) en la subfamilia Helicodontinae, posición que ha sido mantenida por diversos autores hasta la actualidad (Hesse, 1931, 1934; Odhner 1931; Zilch, 1960; Richardson, 1980; Vaught, 1989). Por su parte, los helicondóntidos han sido considerados como una subfamilia de Helicidae (Hesse, 1918; Zilch, 1960; Gittenberger, 1968; Kerney et al., 1983) y también como una familia diferente (Helicodontidae), incluyédolos en la superfamilia Helicoidea (Damianov & Likharev, 1975; Schileyko, 1978) o en Helicondontoidea (Schileyko, 1979).

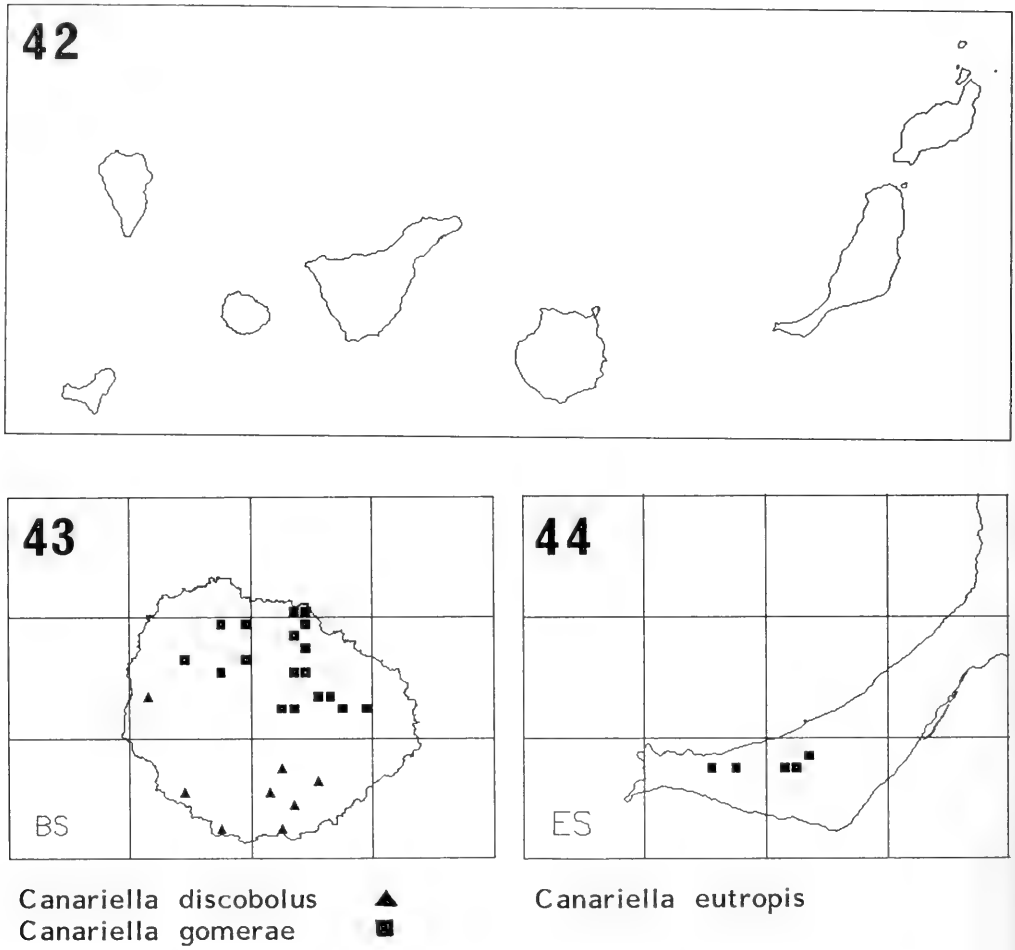
Recientemente, Nordsieck (1987, 1993b) y Schileyko (1991) ubican a *Canariella* entre los higrómidos, que estaban considerados tradicionalmente como una subfamilia de Helicidae; pero, en base a una nueva interpretación de sus caracteres anatómicos, fueron segregados como una familia diferente por Schileyko (1973), opinión compartida por diversos autores (Nordsieck, 1987, 1988, 1993b; Giusti & Manganelli, 1987; Puente & Prieto, 1992).







FIGS. 39–44. Distribución geográfica. Mapas UTM con cuadrículas de 10 × 10 km, realizados según el procedimiento informático de La Roche & Barquín (en prensa). Los símbolos representan cuadrículas de 1 × 1 km; las letras BS, CS, CR y ES designan las cuadrículas de 100 × 100 km. (39–41) Isla de Tenerife. (39) Variedades de *Canariella hispidula;* cuadrado blanco: var. *hispidula;* estrella: var. *bertheloti;* cuadrado negro: var. *fortunata;* triángulo: var. *beata;* círculo: var. *subhispidula;* cruz: var. *lanosa* (la interrupción principal de su área de distribución se debe a la destrucción por el hombre del biotopo natural). (42) Mapa general del Archipiélago Canario (no está hecho a escala). (43) Isla de La Gomera. (44) Isla de Fuerteventura.



FIGS. 42–44.

Los trabajos de Nordsieck y Schileyko han tenido la virtud de reactivar el interés de los malacólogos en la sistemática de los Helicoidea, pero están basados en ideas personales de sus autores, no confirmadas por técnicas objetivas, lo que ha conducido a resultados muy dispares. Nordsieck (1987) coloca a *Canariella* en Ciliellinae (junto con los grupos de *Oestophora* Hesse, 1907, y de *Trissexodon* Pilsbry, 1895, más los géneros *Caracollina* Beck, 1837, y *Ciliella* Mousson, 1872), independiente de Helicodontinae, e incluye a ambas subfamilias en Hygromiidae y a ésta en la superfamilia Helicoidea. Por su parte, Schileyko (1991) incluye a *Canariella* en Ciliellinae (junto con *Ciliella, Haplohelix* Pilsbry, 1919, y *Schileykiella* Manganelli, Sparacio & Giusti, 1989), y a ésta en Ciliellidae, junto con Halolimnohelicinae y Vicariihelicinae; y engloba a Ciliellidae en Hygromioidea, superfamilia no reconocida por Nordsieck (1987, 1993b) y tampoco por Giusti & Manganelli (1987, 1988, 1990) y por Manganelli et al. (1989), mientras que sí lo está por Prieto et al. (1993).

Los caracteres de mayor relevancia utilizados en ambas clasificaciones son los mismos (configuración y posición del aparato estimulador, posición de la bolsa copulatriz con respecto al ovoespermiducto y morfología y posición de las glándulas vaginales), destacando los referentes al aparato estimu-

lador y a la topografía de la bolsa copulatriz. Pero difieren esencialmente por la concepción plesiomórfica que cada una de ellas atribuye a estos caracteres:

- Bolsa copulatriz unida por una banda de tejido conjuntivo y muscular a la pared del pulmón [Schileyko] ↔ bolsa copulatriz situada junto al ovoespermiducto [Nordsieck].
- Con uno (Bradybaenidae, Xanthonychidae) o cuatro sacos del dardo (= estilóforos) [Schileyko] ↔ saco del dardo sencillo [Nordsieck].

Ambas posturas están basadas en una argumentación lógica. Por ejemplo, en relación con la posición de la bolsa copulatriz con respecto al ovoespermiducto, Schileyko (1991), basándose en Fraser (1946) considera que en los pulmonados la bolsa copulatriz se ha formado por la separación de una parte de la cavidad del manto, por lo que la forma inicial pre-helicoide se caracterizó por tenerla unida a la pared del pulmón. Por el contrario, Nordsieck (1987) afirma que la posición de la bolsa copulatriz junto al ovoespermiducto es plesiomorfa, porque se origina a través de su separación del oviducto. Este autor indica que la mayoría de los Stylommatophora, particularmente los más primitivos, la tienen situada de esta forma, y considera que la posición apomorfa (libre) de la bolsa copulatriz podría tener la ventaja de que el contenido (productos de desecho de espermatóforos y esperma) puede ser reabsorbido mejor. También manifiesta que esta posición tiene un origen múltiple evidente dentro de los Helicoidea, porque Bradybaenidae y Helicidae (que la tienen separada del ovoespermiducto) no forman ningún grupo monofilético. Finalmente, en relación con el aparato estimulador, Nordsieck (1985) justifica su opinión señalando que se origina del apéndice del pene de sus antepasados Orthurethra.

Con respecto a la bolsa copulatriz, su origen ovoespermiductal ha sido constatado por Visser (1973) en *Gonaxis* Taylor, 1877, y por Nel (1984) en *Elisolimax* Cockerell, 1893; además, Visser (1977), considera que la bolsa copulatriz de Basommatophora tiene origen diferente que en Stylommatophora; y Visser (1988) y Nordsieck (1993a) llegan a la conclusión de que ambos grupos se separaron muy tempranamente en el proceso evolutivo de los pulmonados, por lo que la evolución de la bolsa copulatriz ha podido seguir caminos diferentes en ellos. Esta posibilidad está avalada, además, por el registro fósil, ya que los Stylommatophora aparecieron en el Carbonífero, alrededor de 150 millones de años antes que los Basommatophora, que lo hicieron entre el Jurásico posterior y el Cretácico (Solem, 1985).

Los géneros aparentemente más próximos a *Canariella* son *Montserratina* Ortiz de Zárate López, 1946, *Ciliella, Schileykiella, Tyrrheniellina* Giusti & Manganelli, 1992 (sinonimia: *Tyrrheniella* Giusti & Manganelli, 1989) y *Ciliellopsis* Giusti & Manganelli, 1990. Estos seis géneros comparten las siguientes características:

(A) Microescultura espiral de la concha formada por crestas muy finas y numerosas.
(B) Ausencia de estilóforos, sacos y apédices accesorios.
(C) El nervio peneano aparentemente se origina del ganglio cerebroideo derecho (no está constatado en *Schileykiella*).
(D) El músculo retractor del ommatóforo derecho pasa entre el pene y la vagina.
(E) Presencia de una vaina envolviendo al pene.

En *Montserratina* la concha tiene una microescultura similar a la de *Canariella.* Con respecto al aparato reproductor, el lugar de inserción del músculo retractor del pene es similar y ambos géneros comparten, por otro lado, el carácter plesiomórfico de presencia de glándulas vaginales digitiformes (de las que carecen los otros géneros), que tienen su porción inicial muy estrecha. La principal diferencia entre ellos consiste en la presencia en *Montserratina* de un pequeño músculo que conecta la vagina con el músculo columelar; además, su papila peneana es perforada típica, aunque el orificio está situado en posición subterminal, y posee una cavidad circular en su pared.

Ciliella tiene la papila peneana muy parecida a la de algunas especies de *Canariella,* habiéndola descrito Manganelli et al. (1989) como una lengua arrugada que delimita a un surco espermático, cuya base abraza completamente a la abertura del epifalo en el pene. Pero posee un lóbulo lateral izquierdo en el collar del manto, carece de glándulas vaginales digitiformes y el músculo retractor se inserta en el límite entre pene y epifalo; conquiológicamente se diferencia, además,

por la presencia de escamas "en forma de uña."

En *Schileykiella* y en *Tyrrheniellina,* como en *Canariella,* la concha tiene costulaciones radiales, además de crestas espirales y pilosidad, y el músculo retractor del pene se inserta en el epifalo. Además, en el pene de *Schileykiella* hay una estructura parecida a la papila de *Canariella planaria,* descrita por Manganelli et al. (1989) como una protuberancia maciza lateral que imita a una verdadera papila peneana. Pero ambos géneros se diferencian claramente de *Canariella* por la ausencia de glándulas vaginales digitiformes.

Ciliellopsis tiene también la microescultura de la concha similar a la de *Canariella,* pero se diferencia de ella por poseer un lóbulo lateral izquierdo del collar del manto, por carecer de glándulas vaginales digitiformes, por la posición del músculo retractor, que se inserta en el límite entre el pene y el epifalo, y por la papila del pene, que es perforada típica, aunque los pliegues del epifalo continuan en su interior (Giusti & Manganelli, 1990: 273, fig. 3C).

Canariella también podría estar relacionada con los géneros *Gasulliella* Gittenberger, 1980, *Caseolus* Lowe, 1852, y *Haplohelix* Pilsbry, 1919, que carecen completamente de estructuras vaginales, según los dibujos de Gittenberger (1980: 207, fig. 5), Mandahl-Barth (1943: lám. 6 y lám. 7, fig. 2) y Verdcourt (1975: 936, figs. 1–6), respectivamente; y, aparentemente, el músculo retractor del pene se inserta en el límite entre el pene y el epifalo, menos en *Caseolus,* en el que parece insertarse en el epifalo. Pero se desconoce en ellos la estructura interna del aparato reproductor, así como la posible presencia o ausencia de papila y de vaina peneana (en *Gasulliella* es muy probable que no exista la vaina peneana, ya que Gittenberger, 1980, no menciona su presencia).

Otros Hygromiidae carecen también de aparato estimulador y sus derivados, pero pertenecen a diversas subfamilias. Por ejemplo, *Ashfordia* Taylor, 1917, y *Szentgalia* Pintér, 1977, están incluídos en Monachinae por la estructura del complejo peneano, similar a la del género *Monacha* Fitzinger, 1833, teniendo además el músculo retractor del ommatóforo derecho libre del pene y de la vagina. *Metafruticicola* Ihering, 1892, *Cretigena* Schileyko, 1972, y *Caucasocressa* Hesse, 1921, han sido incluídos por Nordsieck (1987) en Hygromiinae por la constitución de las vías finales masculinas (con una estructura muy compleja de la región peneana) y por la distribución, iniciándose además el conducto de la bolsa copulatriz casi en el atrio (la vagina es prácticamente inexistente). Otro género que también se podría comparar con *Canariella* es *Cyrnotheba* Germain, 1929, pero carece de vaina envolviendo al pene y tiene diferente anatomía de la papila peneana.

Sin embargo, en una clasificación a nivel superior al del genéro es discutible la validez de los caracteres "A" a "E" anteriormente mencionados, ya que es posible que se hayan originado por convergencia, pues aparecen en otros géneros filogenéticamente distantes (Giusti, pers. com.): el carácter "A" se encuentra también, por ejemplo, en *Xerotricha apicina* (Lamarck, 1822); el "B" también se presenta en *Ashfordia* y *Metafruticicola;* el "C" y el "D" son compartidos por muchos otros géneros de Hygromiidae y, finalmente, sobre el "E" hay pocos datos, pero existen estructuras similares en géneros tan diferentes como *Helicella* Férussac, 1821, y *Helicodonta* (Férussac) Risso, 1826. Otros caracteres probablemente sean más útiles a la hora de buscar afinidades, como los referentes al collar del manto y al complejo paleal; pero tenemos pocos datos sobre ellos en otros géneros, por lo que de momento mantenemos a *Canariella* sin asignar a ninguna subfamilia dentro de Hygromiidae.

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AGE-RELATED DIFFERENTIAL CATABOLISM IN THE SNAIL *BITHYNIA GRAECA* (WESTERLUND, 1879) AND ITS SIGNIFICANCE IN THE BIOENERGETICS OF SEXUAL DIMORPHISM

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ABSTRACT

Catabolic partitioning of carbon and nitrogen was investigated to clarify the sexual dimorphism of bioenergetics in *Bithynia graeca.* Experiments involved post-breeding male and female snails 1, 3, and 11 months old, grazing on Aufwuchs (epiphytic scum flora). Per-snail ingestion and partitioning rates were maximal for 11-month-old snails and declined with age in both sexes. Three-month-old males had lower weight-specific rates and efficiencies than females. Eleven-month-old females had lower weight-specific rates and efficiencies than males. The youngest snails had higher rates in the various components of the energy budget than the older snails.

Key words: Bioenergetics, *Bithynia graeca,* prosobranch snails.

INTRODUCTION

In actuarial (age- and sex-related) bioenergetics, it is generally accepted that the anabolic expenditures of females during the production of eggs or young will far outweigh any comparable effort involved in the production of male gametes. However, this is rarely proved, and particularly in the cases where the sexes do not differ markedly in adult biomass, it is often assumed that the production of eggs in females is compensated for by the greater kinetic expenditure of males (Aldridge et al., 1986). Direct investigation of catabolic partitioning of carbon and nitrogen in individual molluscs has complemented traditional assessment of changing C:N ratios of biomass in molluscan populations (Russell-Hunter & Buckley, 1983) and thus provided a means for actuarial cross-checking or auditing of metabolic efficiencies.

Catabolic allocation can be studied by experiments involving nearly concurrent measurements of oxygen uptake and nitrogenous excretion (Aldridge, 1982; Russell-Hunter et al., 1983; Tashiro, 1982; Tashiro & Colman, 1982). Such investigation can reveal shifts from protein- to carbohydrate-based catabolism. When discussing problems in terms of actuarial bioenergetics, quantification of both physiological rates and ecological efficiencies can be of value in a broadly adaptational approach.

This paper deals with the catabolic partitioning of ingested nitrogen, and protein and nonprotein carbon, in the post-breeding season of the semelparous freshwater prosobranch snail *Bithynia graeca,* in three age-classes—1 month, 3 months, and 11 months old—and in the last two age-classes for each sex. We have examined grazing-feeding in specimens of *B. graeca,* which is an endemic species of Greek lakes; it has been examined for each sex and age class for a population in the artificial Lake Kerkini, Macedonia, Greece. The primary focus of this work was to assess ingestion rates and subsequent bioenergetic partitioning of ingested nitrogen and carbon. Information from feeding and assimilation studies was combined with respiratory and excretory measurements. In assessing differential catabolic allocation, techniques allow distinction between protein carbon and nonprotein carbon in partitioning and provide data that are of wider significance in discussing the complex differential bioenergetics of sexual dimorphism that can occur in this semelparous species.

MATERIALS AND METHODS

Specimens of the prosobranch mollusc *Bithynia graeca* were collected from the dam at the artificial Lake Kerkini in Macedonia, northern Greece. Animals in this population are semelparous and annual and have a

lifespan of approximately 12 months (there are no biennial populations in Kerkini). The sexes are separate, and male and female individuals occur in about the same numbers. Reproduction takes place in spring, and the majority of adults die after egg-laying. Growth rate is quick during spring, and life expectancy decreases with increasing age. *Bithynia graeca* is capable of true tissue degrowth with low mortality rate during the winter, when the snails can remain without food and out of the water for 6 to 7 months because of the management of the lake level (Eleutheriadis & Lazaridou-Dimitriadou, submitted).

In 1992 from May to September, 1-month-old [shell height (H) = 1 mm ± 0.25] (mean ± standard deviation), 3-month-old (H = 1.40 mm ± 0.75) and 11-month-old (H = 4.2 mm ± 0.80) snails from the same population and conditions were brought into the laboratory where they were used in separate experiments on catabolic partitioning, involving nearly concurrent measurements of oxygen uptake and nitrogenous excretion. Their shells were cleaned of Aufwuchs (epiphytic scum flora), and individuals were classified by age and sex. Age determination was made using shell height. Sex determination was based on the male's reddish testis, which was noticeable as a dark area in the last whorls from the apex. The 11-month-old snails were collected in a post-breeding condition. After sorting, snails were placed in water from the Lake Kerkini. All water used in these studies was boiled and filtered prior to the beginning of the study.

After allocation to their respective experimental group, snails were fed with dry Aufwuchs consisting of particles smaller than 50 μ and with a C:N (Carbon:Nitrogen) ratio 7.8: 12.8. Each group, consisting of 15, 10, and 5 individuals from 1-month-old, 3-month-old, and 11-month-old snails respectively, was placed in a culture bowl and fed with a known weight of food over a 48 h period. After 48 h the snails were removed and any uneaten food and faeces were collected from each culture bowl and separated using a filter of 50 μ mesh, and then dried and reweighed. All weights were determined after drying to a constant weight at 60°C. Weights were recorded with a Sauter AR 1014 microbalance (precision ± 0.0001 g).

For each experimental group, batches of food and faeces were analyzed for C and N to determine ingestion and egestion rates. Carbon analyses were carried out using the "wet oxidation" method outlined by Russell-Hunter et al. (1968). Nitrogen analyses were done using the method of D'Elia et al. (1977). The principle of the latter is that nitrogenous compounds in the water are oxidized to nitrate by heating with an alkaline persulphate solution under pressure. These batch analyses provided appropriate C and N values for food and faeces. The weights recorded for each experimental group could then be used to obtain individual rates of total C and N ingestion (TI) and egestion (NA = not assimilated) and, hence total C and N assimilation rates (TA) for each snail (by subtracting the appropriate NA rate from TI)

Oxygen uptake rates were determined using a Digital Oxygen System Model 10 manufactured by Rank Brothers Ltd., Bottisham, Cambridge. The rate of oxygen uptake was calculated from the rate of depletion of oxygen from the water. In no case was this depletion allowed to exceed 40%. The decrease in oxygen tension at the electrode was recorded on a Linseis flat-bed recorder series LS Model 0480L. Controls (in the absence of snails) were run to determine the rate of depletion of oxygen due to the electrode and other possible factors. Measurements on blank chambers (without snails) were carried out before the start of the experiment and after every five determinations.

Young, males and females were placed in groups of 25, 15, and 5 individuals from 1-month-old, 3-month-old and 11-month-old snails, respectively, in 3 ml filtered lake water. In the chamber, the magnetic stirring bar was located under the snails, which were separated from the stirrer by a nylon mesh glued to a plastic ring. Disturbance of the snails appeared to be minimal, as the snails were observed to crawl on the mesh. The mean values of control measurements were subtracted from the percent decrease of O_2 saturation obtained for measurements in the presence of snails. O_2 uptake rates were converted to values for standard temperature, pressure and solubilities using the Standard Methods (American Public Health Association, 1976). Throughout the experiments the temperature of the inlet and outlet water of the waterjacket was maintained at 26°C.

To assess N losses in catabolism, each snail was set up individually in a jar containing 3 ml of water for 24 h at 26°C. After 24 h, the snail was removed and 0.5 ml urease, 15 U/ml (Cooper-Biomedical, Worthington) was

TABLE 1. Tissue dry weight, with per snail rates (micrograms per hour) of ingestion (TI) and assimilation (TA) (mean ± standard deviation) of carbon and nitrogen.

Age (months)	Sex	n	Tissue dry weight (mg)	Total C ingested	Total N ingested	Total C assimilated	Total N assimilated
1	—	51	0.34 ± 0.09	1.74 ± 0.47	0.18 ± 0.07	0.80 ± 0.37	0.10 ± 0.05
3	fem.	33	0.86 ± 0.12	2.34 ± 0.86	0.25 ± 0.08	0.88 ± 0.25	0.12 ± 0.02
3	mal.	39	0.87 ± 0.21	2.00 ± 0.84	0.21 ± 0.08	0.70 ± 0.33	0.09 ± 0.02
11	fem.	26	2.08 ± 0.73	6.36 ± 4.33	0.64 ± 0.38	2.65 ± 1.86	0.28 ± 0.09
11	mal.	23	1.66 ± 0.42	5.79 ± 1.85	0.60 ± 0.15	2.80 ± 1.28	0.32 ± 0.05

added to hydrolyze any urea. The ammonia concentration of the water from each snail was then determined using Berthelot's colour reaction following the modification of Chaney & Marbach (1962). This reaction is based on the formation of the indole dye indophenol blue. Appropriate blanks and standards were used.

After both oxygen uptake and nitrogen excretion rates had been determined, snail shell dimensions were recorded. Snails were then decalcified in 8.5% HNO_3, and the periostracum was removed. The tissue was dried to constant weight at 65°C. This allowed the computation of all data in terms of both rates per individual snail (Table 1, Fig. 1) and as weight-specific values (Table 2).

Comparisons were made between individual rates of ingestion and assimilation (individual data were expressed per milligram of dry tissue) and were further computed so that they were all expressed in carbon terms (as units of protein carbon or nonprotein carbon). The conversion of various partitioning rates into a common carbon currency and the assignment as protein carbon or nonprotein carbon (Russell-Hunter & Buckley, 1983; Russell-Hunter et al., 1983; Aldridge et al., 1985) were based on the following assumptions: that all nitrogen excreted was derived from the breakdown of proteins, and that oxygen was consumed in proportion to the breakdown of organic carbon compounds, both protein and nonprotein.

Weight-specific rates of ammonia excretion can be converted (multiplying by 0.827) to rates of nitrogen excretion. The conversion factor used to estimate the oxygen consumption for protein catabolism from this rate was 5.92 µl O_2/µg N. Thus, a weight-specific rate of oxygen consumption for the protein fraction of catabolism could be estimated. Subtraction of this rate from the overall weight-specific oxygen uptake rates gave the rate of oxygen consumption for the non-

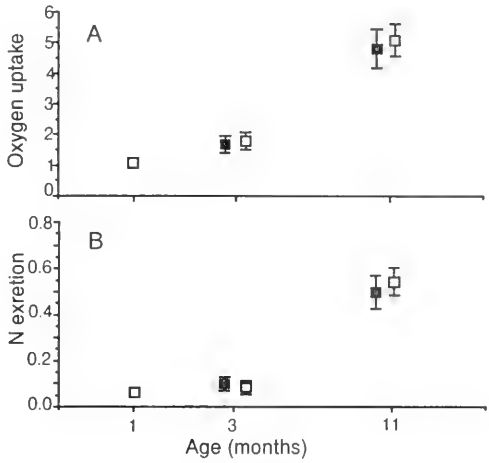

FIG. 1 (A) Oxygen uptake (micrograms O2 per snail per hour) and (B) total nitrogen excretion (micrograms N per snail per hour) for three ages (1, 3 and 11 months) of *Bithynia graeca*. ■, females, □, males. Vertical bars represent standard errors.

protein fraction. Equivalents for carbon mass consumed were derived from the appropriate amounts of CO_2 evolved (0.536 µg C consumed/µl CO_2).

The relation of CO_2 evolved to O_2 consumed differs for proteins and nonproteins. The CO_2 evolved from protein catabolism can be derived directly from the weight-specific nitrogen excretion rate (4.75µl CO_2 evolved/µg N excreted). The CO_2 evolved from nonprotein catabolism can be estimated by multiplying the weight-specific oxygen consumption for the nonprotein fraction by an appropriate respiratory quotient. Food-component analysis for prosobranchs suggests an average of 10% fat and 90% carbohydrate for the nonprotein fraction, giving a respiratory quotient of 0.95. Thus, separate estimates of catabolism (RA = respired assimilation) in carbon terms for protein and

TABLE 2. Mean weight specific partitioning rates (micrograms C per milligram per hour) of protein and nonprotein C sources into the various components of the energy budget.

Age (months)	Sex	n	Protein C ingested	Non-protein C ingested	Protein C assimilated	Non-protein C assimmilated	Protein C in RA	Non-protein C in RA	Protein C in NRA	Non-protein C in NRA
1	—	51	1.70	3.40	0.95	1.42	0.53	0.31	0.42	1.11
3	fem.	33	0.94	1.78	0.45	0.58	0.32	0.28	0.13	0.30
3	mal.	39	0.79	1.50	0.34	0.46	0.25	0.38	0.09	0.08
11	fem.	26	1.00	2.05	0.47	0.79	0.45	0.15	0.02	0.61
11	mal.	23	1.17	2.31	0.63	1.02	0.50	0.18	0.13	0.87

nonprotein could be computed. Rate values were also calculated for total ingestion (TI), egestion (NA) and assimilation (TA) determined in both carbon and nitrogen terms, based on food and faeces, and from these were derived non-respired assimilation (NRA) values. Additionally, all rates (and relative efficiencies) in terms of nonprotein carbon and protein carbon [carbon content being 3.25 times the nitrogen content of protein (Russell-Hunter & Buckley, 1983)] could be computed. In order to find out if rate differences existed among or/and between experimental groups, analysis of variance (ANOVA) and Fisher LSD tests were executed, respectively (Daniel, 1991). Data was logarithmically transformed prior to analysis.

RESULTS

The basic data on catabolic allocation are shown as oxygen uptake and nitrogenous excretion rates in Figure 1. Values for oxygen consumption per individual (Fig. 1A) were statistically higher for 11-month-old snails compared to 3- and 1-month-old snails and for 3-month-old snails in relation to 1-month-old snails ($P < 0.05$). Values for nitrogenous excretion per individual were statistically higher in older than younger snails ($P < 0.05$). Further comparisons made below were weight-specific (i.e., the individual data were expressed per milligram of dry tissue) and were further computed so that data were expressed in carbon terms (as units of protein carbon or nonprotein carbon).

Mean tissue dry weight and individual rates of ingestion and assimilation in C and N terms, for each age and sex fed on Aufwuchs, are shown in Table 1. The females of both ages had slightly higher grazing rates than males, but these were not statistically different. This occurred only for the smaller females for the assimilation rate but not for the older females. The older snails had higher ingestion and assimilation rates for C and N than the younger snails ($P < 0.05$).

For each age and sex that fed on Aufwuchs, mean weight-specific rates of protein and nonprotein carbon partitioning are shown in Table 2. Rates for males and females were compared using ANOVA and Fisher LSD test (Table 3).

Overall, young females had higher weight-specific rates of carbon (ingested and assimilated) than young males. In contrast, older males generally had higher weight-specific rates than females in the same age-class. The weight-specific carbon partitioning rates tended to decline with age from 1-month to 3-month-old snails only, and increased from 3-month-old to 11-month-old snails. The differences were statistically significant between the 1-month-old snails and the other age classes for protein C ingested ($P < 0.05$) (Table 3), between 1-month-old and 3-month-old male snails for protein C assimilated ($P < 0.05$) (Table 3) and between 1-month-old and 3-month-old snails for nonprotein C ingested ($P < 0.05$). There were no statistical differences for nonprotein C assimilated. The differences were significant between 11-month-old male and 3-month-old male and between 1-month-old and 3-month-old snails for protein RA ($P < 0.05$) (Table 3).

Categories of carbon partitioning rates per snail are shown in Figure 2. In Figure 2A ingested and assimilated values are contrasted, whereas Figure 2B presents the partitioning between respired and nonrespired assimilation. Expressed in units per snail and time, all rates were highest in 11-month-old snails. Differences could be detected in both rates of ingestion and in the differential catabolism of protein and nonprotein substrates. The protein carbon efficiencies in

TABLE 3. Comparisons between different age and sex groups (1-month-old, 3-months-old or 11-month-old female (F) or male (M) snails) of *Bithynia graeca* concerning the protein C ingested, protein C assimilated and protein C in respired assimilation with Fisher LSD test (*:P < 0.05).

			1	3 F	3 M	11 F	11 M
		Protein C ingested (PCi)	—				
		Protein C	—				
1		assimilated (PCa)					
		Protein in R.A. (PRA)	—				
	M	PCi	0.174*	0.190	—		
	M	PCa	0.334*	0.366	—		
3	M	PRA	0.395*	0.257	—		
	F	PCi	0.174*	—			
	F	PCa	0.334*	—			
	F	PRA	0.390*	—			
	M	PCi	0.163*	0.180	0.180*		—
	M	PCa	0.313	0.347	0.347		—
11	M	PRA	0.385	0.211	0.251*		—
	F	PCi	0.163*	0.180	0.180	—	0.170
	F	PCa	0.313	0.347	0.347	—	0.327
	F	PRA	0.386	0.243	0.249	—	0.235

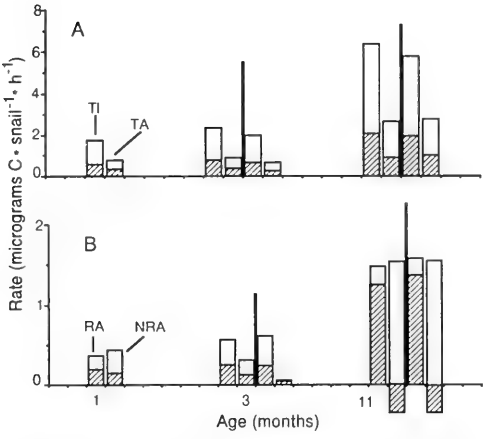

FIG. 2. Partitioning of nonprotein (□) and protein carbon (▨) of *Bithynia graeca*. (A) Total ingestion (TI) and total assimilation (TA). (B) Respired assimilation (RA) and nonrespired assimilation (NRA). The left pair of histogram bars are for females; the right pair, for males.

non respired assimilation for 11-month-old snails are negative indicating that protein degrowth takes place in order to meet the needs of reproduction knowing that efficiencies tend to decline with age. Females had higher ingestion rates for protein carbon than males in both age classes, but these were not statistically different. The values for protein C ingested and assimilated were derived from N ingested and assimilated, so the results of the statistical tests were the same. Statistical differences were detected in rates of ingestion in differential catabolism of nonprotein substrates between 11-month-old snails and all the other age-classes ($P < 0.05$), and in rates of assimilation of nonprotein substrates between 11-month-old snails and the 3-month-old males and 1-month-old snails ($P < 0.05$). Protein RA were statistically different between 11-month-old snails and all the other age-classes.

Average assimilation efficiencies are shown in Table 4. Overall, young females had higher assimilation efficiencies than young males, while in contrast, older males had higher efficiencies than older females. The youngest snails had higher assimilation efficiencies than 3-month-old and 11-month-old female snails.

The differences in processing rates with age and sex noted above could be expressed as percentage values of nonprotein assimilation over total ingestion (gross efficiencies) or over total assimilation (net efficiencies) (Russell-Hunter & Buckley, 1983). For each age and sex, average gross and net efficiencies are presented in Table 5. Young females had higher gross and net growth efficiencies than

TABLE 4. Mean assimilation efficiencies (percent) for various budget components.

Age (months) Sex	1	3 females	3 males	11 females	11 males
n	51	33	39	26	23
C assimilation efficiency	45	38	33	42	48
Protein C assimilation efficiency	57	48	46	47	54
Nonprotein C assimilation efficiency	42	33	31	39	44

TABLE 5. Gross and net growth efficiency averages (percent).

Age (months)	Sex	n	Gross growth efficiency			Net growth efficiency		
			Total C	Protein C	Nonprotein C	Total C	Protein C	Nonprotein C
1	—	51	30	25	32	64	04	78
3	fem.	33	16	14	17	42	20	52
3	mal.	39	08	14	05	23	30	17
11	fem.	26	21	02	30	50	30	77
11	mal.	23	29	11	38	60	44	85

young males and the opposite occured in older snails. The youngest snails had higher gross and net efficiencies for total C than all the other age classes.

DISCUSSION

During a lifespan of 12–13 months, *B. graeca* reach up to 7 mm in length and are active from March to October. The active period consists of three parts: breeding (April–June), post-breeding (July–August) and prewinter period, lasting from September until the onset of diapause (Eleutheriadis & Lazaridou-Dimitriadou, 1992). In this study, it was hoped that age- and sex-related differences in catabolic allocation would be revealed. *Bithynia graeca* feeds both by radula grazing on detritus and Aufwuchs and by ctenidial filter-feeding on phytoplankton and other suspended organic material, as any other species of Bithyniidae. The frequency of filter-feeding may depend on the availability of other food sources and the nature of the suspended particles (Fretter & Graham, 1962).

The prediction of sexual dimorphism in the metabolic processes of animals involves higher anabolic rates and efficiencies for females and higher catabolic rates and efficiencies for males. The relatively higher anabolic expenditures of females are invested in the production of eggs or young (Russell-Hunter & Buckley, 1983), and it is usually assumed that in males the greater kinetic energy expenditures are directed to extensive gene dispersion.

It must be noted that the data for *B. graeca* were presented both as rates (individual and weight-specific) and as efficiencies (gross and net), and were derived from experiments limited to the grazing mode of feeding (excluding the alternative filter-feeding mode) and to post-breeding snails (excluding the spring period of exponential growth in females).

The age- and sex-related differences in catabolic allocation revealed the theorized trade-offs against the known bioenergetic differences in rates and efficiencies between the sexes. Oxygen consumption and nitrogenous excretion in older snails were statistically higher than those in younger snails, and this also occured for TI and TA in both carbon (protein and nonprotein) and nitrogenous terms.

The youngest snails had higher rates in the various components of the energy budget than all the others. The reason for these differences could be the decrease in metabolic rhythm as the snails were growing up. This has also been observed in terrestrial gastropods (Jennings & Barkham, 1976; Lazaridou-Dimitriadou & Daguzan, 1978; Stern, 1968; Zeifert & Shutov, 1979; Charrier & Daguzan, 1980; Staikou & Lazaridou-Dimitriadou, 1989; Lazaridou-Dimitriadou & Kattoulas, 1991) and in freshwater gastropods (Aldridge et al., 1986).

Overall, young females had higher average assimilation efficiencies than young males because the anabolic expenditures for the production of eggs would probably far outweigh any comparable effort involved in the production of male gametes. In contrast, 11-month-old males had higher assimilation efficiencies than 11-month-old females, probably because the males had greater kinetic expenditures than females. This species is semelparous and lives for 12 to 13 months, so the 11-month-old snails probably had lower energy expenditures in the production of gametes. For the same reasons, young females had higher gross and net growth efficiencies than young males and the opposite in older snails.

The ratio N-RA/TA in carbon terms (net growth efficiency) ranged from 23–64% for *B. graeca.* Hunter (1975) gave almost the same range (22–41%) for two populations of *Lymnaea palustris,* whereas McMahon (1975) reported 22–52% for four generations in three populations of *Laevapex fuscus.* In two populations of the European stream limpet *Ancylus fluviatilis* much lower values (11.2% and 11.8%) were found (Streit, 1975, 1976a, cited in Russell-Hunter & Buckley, 1983). Burky (1971) reported 19% for N-RA/TA in *Ferrissia rivularis,* and Aldridge (1982) reported 12.2%, 12.4% and 12.9% in carbon terms for three populations of *Leptoxis carinata.* Tashiro & Colman (1982) reported the highest values (more than 90%) for N-RA/TA in *Bithynia tentaculata* for filter-feeding. The effect of food quantity and quality must be the reason for these many-fold differences in net growth efficiencies as McMahon et al. (1974), McMahon (1975), and Aldridge et al. (1986) have also noted.

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DIETARY PREFERENCE OF THREE FRESHWATER GASTROPODS FOR EIGHT NATURAL FOODS OF DIFFERENT ENERGETIC CONTENT

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ABSTRACT

The food preferences of three freshwater gastropods, *Radix peregra* (Pulmonata), *Bithynia tentaculata* and *Bythinella dunkeri* (Prosobranchia), have been examined in laboratory experiments. Eight natural foods, a green alga, a cyanobacterium, and leaves of sycamore maple, alder and oak with different conditionings, were tested in various combinations. The number of animals found on these foods during a three-hour period was counted.

There were significant differences in the food choice of the three species: *Bithynia tentaculata* clearly preferred algae and cyanobacteria, *Bythinella dunkeri* selected leaves conditioned by microorganisms and discriminated against unconditioned leaves and algae, and *Radix peregra* showed intermediate food preferences for almost all foods.

For *Radix* and *Bithynia,* the results of these experiments were correlated with the C:N ratio and the % nitrogen content of the food items: preference increased with decreasing C:N ratios and with increasing nitrogen content.

There was no such correlation for *Bythinella.* The possible reasons for this are discussed.

Key words: Optimal foraging, food choice, natural food, C:N-ratio, freshwater gastropods, *Radix, Bithynia, Bythinella.*

INTRODUCTION

One of the assumptions of optimal foraging theory is that "high fitness is achieved by a high net rate of energy intake" (Begon et al., 1990).

If they are behaving as optimal foragers, grazing gastropods should therefore feed preferentially on foods of high energy content when presented with an array of potential food sources of equal quantity and accessability.

Discrimination by gastropods according to algal class (Calow, 1970; Imrie et al., 1990), food particle size (Levinton & DeWitt, 1989) and even taste (Daldorph & Thomas, 1988) has been described. In a related paper (Brendelberger, 1992), I found that various components of natural food were contrastingly attractive to *Radix peregra* and *Bythinella dunkeri.* McMahon et al. (1974) was able to relate the uptake of periphyton and the rejection of macrophyte tissue by gastropods to the carbon to nitrogen ratios of these two foods, low and high respectively. Despite the success of that study, a systematic investigation of a wide array of natural gastropod foods and their energy value has not yet been made.

Using a true food preference method (*sensu* Peterson & Renaud, 1989), I therefore tried to relate the food preferences of three freshwater gastropod species, *Radix peregra, Bithynia tentaculata* and *Bythinella dunkeri,* to the C:N ratios and the %-nitrogen content of the foods under consideration.

MATERIAL AND METHODS

The experiments were made with three freshwater gastropod species, *Radix peregra* (Müller) (Pulmonata, Lymnaeidae), *Bithynia tentaculata* (L.) (Prosobranchia, Bithyniidae), and *Bythinella dunkeri* (Frauenfeld) (Prosobranchia, Hydrobiidae). *Radix* and *Bithynia* were collected in shallow waters near the shore of the Rhine near Köln, whereas *Bythinella* was from a first order stream 30 km east of Köln, Germany.

The animals were kept in the laboratory under constant conditions, a 12:12 h light-dark cycle at 18±2°C, for at least two months before the experiments began. During this acclimation period, they were fed with lettuce (*Radix, Bithynia*) or *Fontinalis antipyretica* (a moss) (*Bythinella dunkeri*) in non-limiting amounts.

Food choice was tested in glass petri dishes, 22 cm diameter for *Radix* and *Bithynia,* 6 cm diameter for *Bythinella,* using ten animals per dish. Foods were always offered in pairs at opposite sides of the experimental dishes. The position of the animals in the dishes was recorded every ten minutes during the three-hour experimental period. The "preference" of a test food was calculated from the sum of all animals feeding on that food during the experimental period.

The foods offered were a cyanobacterium (*Synechococcus elongatus*), a green alga (*Chlamydomonas reinhardii*) and leaves of three deciduous tree species with different conditioning. Leaf discs of sycamore maple (*Acer pseudoplatanus*), alder (*Alnus glutinosa*) and oak (*Quercus robur*) were conditioned for 10 days. Additional food sources were watered alder leaves or ultrasonicated leaves (i.e. depleted of the microorganisms growing on the decaying leaf surface), or the microorganisms from the leaf surface.

For details of the experimental set-up and preparation of the different foods, see Brendelberger (1992).

In order to obtain realistic results bearing as much similarity to field conditions as possible, the following details were observed:

- Some species of freshwater gastropods, such as *Bithynia tentaculata,* show a circannual rhythm of activity, even under constant laboratory conditions (Brendelberger & Jürgens, 1993). Therefore, all preference tests were performed in spring and summer when all species were active.
- Food choices of freshwater gastropods are not constant, but may change over time or with the age of the animals (Skoog, 1978; Egonmwan, 1991). For the experiments, only juveniles of *Radix peregra* (6–8 mm shell length) and *Bithynia tentaculata* (5–6 mm shell length) were used. Juveniles of *B. tentaculata* have been shown to have higher growth rates than adults (Tashiro, 1982). They should therefore search for food more constantly and intensely. The juvenile animals of *Radix* and *Bithynia* never showed any interactions that could interfere with the search for food. The maximum body length of *Bythinella dunkeri* is only 3 mm; therefore, adult animals were used for their food preference tests.
- Gastropods may feed preferentially on food items that they have been eating before (Bleakney, 1989; Imrie et al., 1990). Therefore, the maintenance food given during the acclimation period was excluded from the experiments.
- Food preference has been found to change with hunger level (Calow, 1973). The effect of hunger level on food preference was tested with *Radix peregra*. A moderate hunger level was created for all species in all experiments.
- As gastropods react not only to the kind of food, but also to the quantity of food offered (Daldorph & Thomas, 1988; Madsen, 1992), all food items were given at similar, comparable concentrations. Algae and cyanobacteria were fed at identical biovolumes of 1.2×10^9 fl per filter, and leaf discs had an area of 5 cm^2 (big petri dishes) or 1 cm^2 (small petri dishes).
- Food was replaced by fresh food of the same kind and concentration every hour. This had two effects: food could not be depleted by the animals, i.e. it did not lose its attractivity, and the animals had to find and actively crawl on the food several times in order to produce high preference values.
- Animals found to be inactive, i.e. not having changed their position during the first ten minutes of an experiment, were replaced by other animals of the same species and hunger level.
- All food combinations were tested with 3–5 replicates of ten animals each per species.

In control experiments without food or with empty filters only, the homogeneity of the distribution of the animals was tested with chi-square statistics ($p < 0.05$). The experiments with detritus and algae were designed as true food preference experiments, *sensu* Peterson & Renaud (1989). As they recommended, when there is no depletion of food, t-tests for pairwise comparisons of the experimental results were carried out.

An elemental analysis was performed in order to characterize the nutritional value of each food: aliquots (±5 mg) of each food were combusted in a Carlo-Erba elemental analyser, and carbon and nitrogen content were recorded. The results were related to the food preference values found for the three gastropod species.

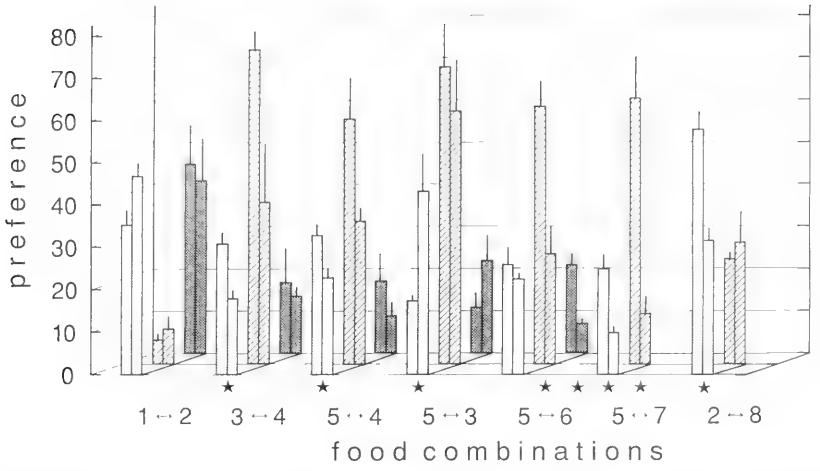

FIG. 1. Pairwise results (mean ± SD) of food preference experiments for three gastropod species and 8 food items; dotted (front): *Radix peregra,* dashed (medium): *Bythinella dunkeri,* dark grey (back): *Bithynia tentaculata*; 1 = *Synechococcus elongatus*; 2 = *Chlamydomonas reinhardii*; 3 = Sycamore, conditioned; 4 = oak, conditioned; 5 = alder, conditioned; 6 = alder, ultrasonicated; 7 = alder, watered; 8 = microorganisms from alder; * : food choice of a species is significantly different for pairwise tested foods at $p < 0.05$; Vertical bars representing Standard deviations are scaled to half the SD values.

RESULTS

The results of food preference tests are influenced by the hunger level of the animals; well-fed animals are more selective than hungry ones (Calow, 1973). Hungry snails show high activity searching for food and are thought to discriminate less between food items of different quality. The effect of hunger level has been tested with *Radix peregra*. Four sets of 3 × 10 animals each were starved for 1, 2, 4 or 7 days. After this time, the attractivity of *Chlamydomonas* cells at standard concentration was tested. The attractiveness increased from 11.5 ± 5.8 (animals found on this food per experimental period of 3 hours) after a one-day hunger period, to 22.3 ± 10.4 (2 days), 40.8 ± 8.7 (4 days), and 55.0 ± 5.2 (7 days). After even longer periods, animals were found to behave very erratically: some showed higher activity, while others completely ceased moving around. The intermediate hunger level of 4 days was therefore chosen for the experiments.

Without food or with only clear filters, *Radix, Bithynia* and *Bythinella* were homogeneously distributed in their petri dishes. But when natural food was offered, the animals showed significant differences with respect to different foods, the three gastropod species behaving differently (Fig. 1). *Radix peregra* had preference values from 9.8 for watered alder leaves to 58.2 for *Chlamydomonas* cells. Medium attractivity was for conditioned leaves of alder, sycamore and oak, for *Synechococcus* and for microorganisms isolated from alder. Leaves deprived of their microorganisms, i.e. ultrasonicated leaves, were less attractive. *Bythinella dunkeri,* in contrast, was not attracted by algae and cyanobacteria: *Chlamydomonas* and *Synechococcus* both gave preference values well below 10. Very high attractivity was shown to incubated leaves, with no statistical difference between alder, sycamore and oak. However, when watered leaves or ultrasonicated leaves were presented, their attractivity was much lower than that of the conditioned leaves. *Bithynia tentaculata,* the third species, preferred *Chlamydomonas* and *Synechococcus* much more than (2 × greater) any kind of leaves.

In general, *Radix peregra* fed on almost all foods at intermediate values, *Bythinella dunkeri* clearly preferred detritus and discriminated against algae and cyanobacteria, whereas *Bithynia* did just the opposite: it preferred algae and cyanobacteria and discriminated against detritus.

The results of the elemental analysis are presented in Table 1. The C:N ratio increased from about six for cyanobacteria and algae to

TABLE 1. Carbon to nitrogen ratios and nitrogen content (percent) of eight food types (mean ± SD; all determinations in triplicate):

No	Food type	C:N	%N
1	*Synechococcus elongatus*	5.4 ± 0.1	8.0 ± 0.9
2	*Chlamydomonas reinhardii*	6.0 ± 0.9	7.3 ± 3.9
3	Sycamore maple, incubated	18.8 ± 5.0	2.5 ± 0.3
4	Oak, incubated	24.4 ± 0.8	2.0 ± 0.3
5	Alder, incubated	12.4 ± 0.6	3.9 ± 0.2
6	Alder, ultrasonicated	14.3 ± 0.8	3.5 ± 0.2
7	Alder, watered	15.4 ± 0.3	3.2 ± 0.1
8	microorganisms from alder	12.2 ± 1.2	2.9 ± 0.6

a maximum value of 24.4 for incubated oak leaves. Intermediate values were found for incubated alder and sycamore leaves, for watered and ultrasonicated leaves of alder, and for microorganisms from alder. As the carbon content of these foods was generally fairly constant (between 43% and 48% of dry weight), changes in C:N ratios reflect changes in nitrogen content. This is shown in the fourth column of Table 1. Absolute values were from 2% to 8% nitrogen, with a minimum of 2% for oak and a maximum of 8% for cyanobacteria.

As food with a low C:N-ratio and/or a high nitrogen content is generally considered to be better food (McMahon, 1974), I tried to correlate these data with the results of the food preference experiments. Figure 2 shows that there is a significant correlation between C:N ratio and food preference (solid line) for *Radix peregra* and for *Bithynia tentaculata*. The corresponding regressions are:

Radix peregra: $y = 21.421 - 0.248x$; $n = 14$; $r = -0.532$; Bithynia tentaculata: $y = 22.554 - 0.375x$; $n = 14$; $r = -0.722$; (with y = C:N-ratio and x = food preference value).

The nitrogen content of these foods was also significantly correlated with food preference (Fig. 2, dashed line):

Radix peregra: $y = 1.190 + 0.096x$; $n = 14$; $r = 0.615$; *Bithynia tentaculata*: $y = 1.037 - 0.143x$; $n = 14$; $r = 0.876$; (with y = %N-content and x = food preference value).

The food preferences of *Bythinella dunkeri*, on the other hand, could not be related to either C:N-ratio or nitrogen content. Therefore, no lines have been drawn for this species in Figure 2. The possible reasons for this are discussed below.

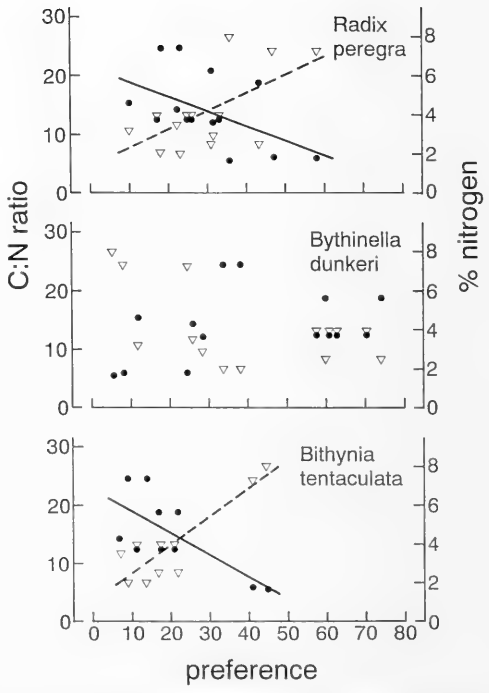

FIG. 2. Relationships between food preference and C:N-ratio (dots, line) and between food preference and % nitrogen content (triangles, dashed) for *Radix peregra, Bythinella dunkeri* and *Bithynia tentaculata*. Each symbol represents the mean of 3–5 replicates with ten animals each. For details of linear regressions, see text.

DISCUSSION

Feeding is one of the main interactions between an animal and its environment. If the quantitative and qualitative aspects of this process are precisely known, the animal's position in the food web of its habitat can be evaluated.

The first step in feeding is the selection of suitable food items. For freshwater gastropods, this behaviour is governed by chemical and tactile stimuli (Masterson & Fried, 1992). Therefore, investigations of the attractive or

deterrent properties of isolated substances, such as amino acids, sugars or phenolics (Norton et al., 1990), are helpful, but cannot be used directly to explain the performance of the animals in the field.

I therefore concentrated upon different natural foods, detritus and its components. The results show that there are significant differences in the behaviour of the three snail species tested and also in their responses to different foods.

Radix peregra showed intermediate preference values for many foods, with a slight preference for green algae. This is in accordance with previous studies, in which *Radix peregra* was shown to feed preferentially on green algae (Calow, 1973a,b; Knecht & Walter, 1977; Lodge, 1986; Brendelberger, 1992), although in general, pulmonates are regarded as generalist herbivores (Madsen, 1992).

Bythinella dunkeri, in contrast, selected strongly against algae, but preferred conditioned sycamore maple and alder leaves. This has also been observed in previous experiments (Brendelberger, 1992). A preference for conditioned rather than unconditioned leaves has also been found for *Gammarus pulex* and *Asellus aquaticus* by Bärlocher (1990). A possible explanation for this phenomenon is the increased availability of amino acids produced by aquatic hyphomycetes during conditioning.

Bithynia tentaculata preferred green algae and cyanobacteria. This can be explained by *Bithynia*'s tendency to feed on suspended food, mainly algae, whenever possible (Brendelberger & Jürgens, 1993). Filtration of the green alga *Chlorella* has been shown to yield a higher net gain of carbon and nitrogen per respired cost than grazing (Tashiro & Colman, 1982). Consequently, the uptake of filterable green algae should be favoured whenever possible. The various detrital components had almost no attractivity for *Bithynia*.

Thus, it can be shown that these three species, even though two of them are from the same habitat, behave completely differently, and that they clearly discriminate between different food items.

In the process of conditioning, bacteria and aquatic fungi degrade the organic parts of deciduous leaves. The maximum biomass of fungi on elm and oak leaves occurs in the second week (Findlay & Arsuffi, 1989). For the present study, leaves were conditioned for ten days. Aquatic hyphomycetes increase the protein and nitrogen content of leaves (Kaushik & Hynes, 1971), whereas Findlay & Arsuffi (1989) found that the biomass of conditioning microorganisms may equal 5.2% of the leaf biomass in terms of carbon. Therefore, carbon and nitrogen seem to be good indicators of the total energy content (C) and the share of easily available substances (N) (Aldridge, 1983), and they are suitable measures to determine energy fluxes through individuals or populations (Russell-Hunter & Buckley, 1983). Numerical values for these are given by Richardson (1990): he found a C:N-ratio of 19.4, 2.4% nitrogen in alder leaves. The alder leaves in this paper had C:N ratios of 18.8 and 2.5% nitrogen. Values for ash, which is less attractive to shredders (Richardson, 1990), are 35.6 (C:N) and 1.2% nitrogen. Oak leaves are known to degrade more slowly than sycamore leaves (Kaushik & Hynes, 1971); therefore, the microorganismal biomass on the leaves after ten days will probably be different, contributing to their contrasting attractivity.

The green alga *Chlorella,* used successfully in feeding experiments with *Bithynia tentaculata* by Tashiro & Colman (1982), was found to have a low C:N-ratio of 12.0 and 4.6% nitrogen. These values are even surpassed, in terms of food quality, by *Chlamydomonas reinhardii* used in this study (C:N = 6.0; 7.3%N; cf. Table 1).

The food preference of *Radix peregra* and *Bithynia tentaculata* could indeed be explained by the C:N-ratios and nitrogen content: food preference increased with increasing nitrogen content and with decreasing C:N-ratio. A preference for food rich in nitrogen has also been found by Newman et al. (1992) for an amphipod, a caddisfly, and a physid snail.

The food preference of *Bythinella dunkeri,* however, cannot be explained by carbon and nitrogen content. There are several possible explanations for this. In contrast to *Radix* and *Bithynia,* in which juvenile animals were tested, adult *Bythinella* had to be used because of the species' small size. But animals do not only select food that is of generally high quality, they also select food items to meet specific requirements. These requirements may differ for adult *Bythinella,* which may be investing more energy in reproduction, whereas juvenile *Radix* and *Bithynia* are investing in somatic growth. A switch in preference from juvenile to adult snails, caused

by changing specific requirements during an animal's life history (Tashiro, 1992), is a possible explanation.

In previous experiments, *Bythinella* showed strong preference for diatoms (Brendelberger, 1992). Diatoms (not tested here) are characterized by their frustules that consist mainly of silica. Their carbon content per unit weight is known to be lower than in green algae (Calow, 1970). Thus, *Bythinella* may not get the appropriate "cues" of good food when fed green algae and detritus only. Alternatively, tactile stimuli may be more important for this animal, which occurs in fast-flowing, low-order streams. The importance of contact chemoreception has been shown for *Ancylus fluviatilis* (Calow, 1973), a snail occurring in the same kind of habitat as *Bythinella. Radix* and *Bithynia,* in contrast, may be guided predominantly by distant chemoreception.

Assuming that good food is characterized by a low C:N-ratio and a high nitrogen content per unit biomass, *Radix peregra* and *Bithynia tentaculata* were found to be optimal foragers. *Bythinella dunkeri,* in contrast, did not show optimal foraging based on these criteria. But these two variables, C:N-ratio and nitrogen content, are but two of many ways of characterizing natural foods. At certain times, trace element content, vitamins, essential amino acids, and other factors may be more important for an animal than overall organic content alone.

It remains to be tested whether the foods thus selected and eaten by the snails are also those that can be better assimilated.

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PATTERNS OF LAND SNAIL DISTRIBUTION IN A MONTANE HABITAT ON THE ISLAND OF HAWAII[1]

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ABSTRACT

A quantitative survey of a 35 km^2 area between 1,500 m and 2,100 m elevation on the island of Hawaii recorded at least 16 species of land snails. Fifteen of these are probably endemic to the island; one is indigenous but not endemic. Canonical correspondence analysis (CCA) of their local distributions in relation to substratum (i.e., lava) type, altitude, and a suite of vegetation-related variables, explained 24% of the variance in distribution and abundance. The unexplained variance is probably related to a range of other abiotic, biotic, stochastic and scale factors. Of this 24% overall variance, 79% was explained by axes 1 and 2 of the CCA, which seemed related most strongly to lava type and altitude, respectively. The vegetation-related variables seemed relatively unimportant, although there was a hint that a number of species were negatively associated with the plant community characterized as "*Dodonaea* shrubland." Military activities, the presence of introduced feral ungulates, and the increasing trend of invasion by non-native plants, all have the potential to damage this unique fauna.

Key words: land snails, Hawaii, ecology, conservation, distribution patterns, canonical correspondence analysis.

INTRODUCTION

The native land snail fauna of the Hawaiian Islands, with 779 recognized species (Cowie et al., in press), is one of the most speciose in the world per unit area (cf. Solem, 1984). Systematic monographs of most of the groups represented are available, but there remain great difficulties in dealing with the fauna because many of these works adopted a now outdated, essentially conchological species concept, which led to considerable overdescription of taxa. In addition, and despite this intensive study of some groups, a number of groups remain much less well known, with large numbers of undescribed species (e.g., the Endodontidae: Solem, 1976, 1990). Apart from the notable studies by M. G. Hadfield and his colleagues on the growth, demographics and population dynamics of certain species of Achatinellinae (Hadfield & Mountain, 1981; Hadfield, 1986; Hadfield & Miller, 1989; Hadfield et al., 1993), undertaken largely with a view to their conservation (see also Severns, 1981), virtually nothing is known of the ecology of the Hawaiian land snails, except what can be gleaned from scattered anecdotal comments in the taxonomic literature.

The present study was initiated as a simple inventory survey (Cowie & Nishida, 1993), part of a wider environmental impact assessment that also included surveys of plants and vertebrates. It is the first study to survey the entire malacofauna of a particular area in the Hawaiian Islands, rather than to focus on particular taxa. It is also the first time distributions of snails in the Hawaiian Islands have been assessed quantitatively in relation to habitat characteristics. Local patterning in the land snail distributions was analyzed by canonical correspondence analysis (Ter Braak, 1986) in relation to altitude, substratum (i.e., lava) type, and to a range of vegetational variables derived from other parts of the wider environmental assessment.

MATERIALS AND METHODS

The Study Area

The study took place on the island of Hawaii, the largest and youngest island in the Hawaiian chain (Armstrong, 1983; Clague & Dalrymple, 1987). The study area (Fig. 1) is located in the saddle area between the two largest volcanoes, Mauna Loa and Mauna

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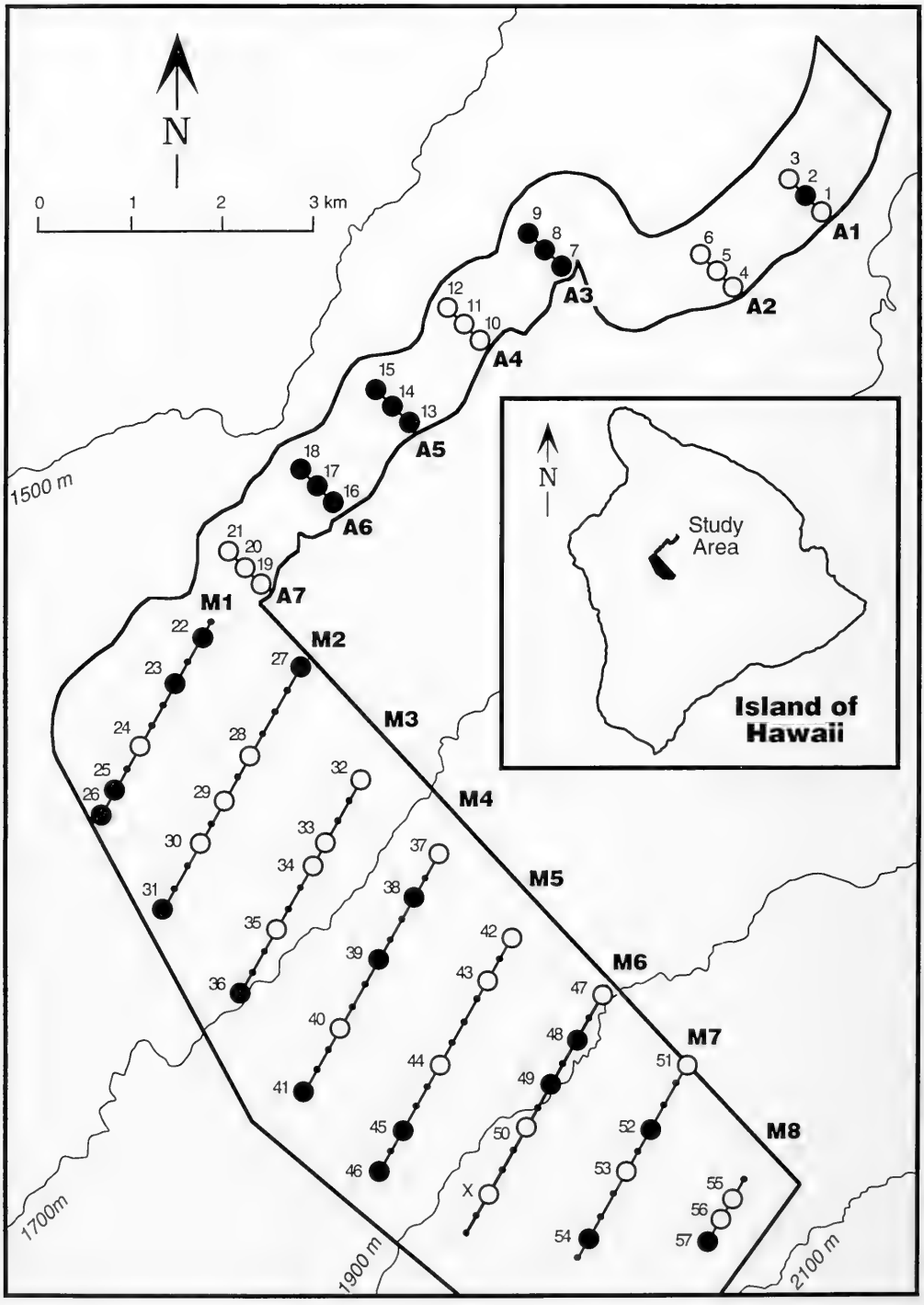

FIG. 1. Map of the study area showing the location of transects and sampling sites and the type of lava at each site that was surveyed for snails. Open circles — pahoehoe; closed circles — aa.

Kea, just on the leeward (west) side of the saddle and on the slopes of Mauna Loa. It is considered in two parts: the main area and the approach road area, comprising a total of about 35 km^2. It slopes gently from an altitude of approximately 1,500 m to 2,100 m, with a northwesterly aspect. Rainfall is low, around 500 mm/yr, and varies little over the study area, although declining somewhat with increasing altitude (Armstrong, 1983; Giambelluca et al., 1986). Mean annual temperature on the island of Hawaii decreases with increasing altitude at a rate of approximately 5.5°C/1,000 m (Armstrong, 1983), giving a range from about 16°C to 13°C from the lower to the upper parts of the study area. The study area is criss-crossed by lava flows from Mauna Loa, some relatively recent and as yet essentially unvegetated, others older and with established vegetation. Most of the study area falls within a "kipuka," the "Kipuka Alala," which is an area of climax vegetation that has not recently been covered with lava, although a number of relatively recent and virtually unvegetated lava flows do extend into the study area. Hawaiian lava is of two main types (Peterson & Tilling, 1980), known by their Hawaiian names, that conceivably offer different habitat characteristics for snails: smooth "pahoehoe," derived from rapidly flowing lava, and jagged, broken "aa." Both types are present in the study area (Fig. 1). The vegetation is montane and subalpine dry shrubland and forest (Gagné & Cuddihy, 1990). Four main vegetational communities were recognized (modified from Gagné & Cuddihy, 1990): (1) bare lava or very sparse pioneer vegetation, (2) *Metrosideros* forest (canopy dominated by *Metrosideros polymorpha* Gaud. [Myrtaceae]), (3) *Sophora/Myoporum* forest (canopy codominated by *Sophora chrysophylla* (Salisb.) Seem. [Fabaceae] and *Myoporum sandwicense* Gray [Myoporaceae]), and (4) *Dodonaea* shrubland (low canopy dominated by *Dodonaea viscosa* Jacq. [Sapindaceae]).

The study area is part of a military training area. It has been modified locally by construction of military facilities, including roads, buildings, targets, and flattened areas surfaced with crushed lava. Miltary activity in the area has probably also fostered the introduction of non-native weeds, especially grasses (Gagné & Cuddihy, 1990). In addition, non-native feral ungulates have probably had a significant impact on the constitution of the plant communities. Nevertheless, the plant communities, particularly the trees and shrubs, remain essentially native.

Sampling Sites

The present study was part of a broader zoological and plant community assessment, for which a grid of transects 1.2 km apart was established. These transects were designated M1–8 in the main area and A1–7 in the approach road area (Fig. 1). A total of 104 sites were located and flagged at 250 m intervals along these transects. All 21 sites in the access road area (sites 1–21 of the present study) and 37 of the 83 sites in the main area (sites 22–57, plus an additional site "x"—see below) were sampled for land snails (Fig. 1). Selection of sampling sites for snails in the main area was designed to give a relatively even rather than random coverage of the area. None of the sampled sites was located in the immediate vicinity of an area highly modified by military construction.

Sampling Protocol

Field work took place between 8 March 1992 and 9 February 1993. Two samples, collected by searching through litter and soil, often in, around and under rocks (turning them over or removing them, especially in areas of aa lava), were taken at each of the 58 sampling sites (except one site on transect M6—site "x"—where only one sample was taken, and four sites—sites 15, 16, 31, 57—where no snails were found). Each sample was taken by one person working for 1 h, having identified appropriate habitat within 10 m of the flagged site. Although there may be unconscious differences in sampling strategy between collectors, sampling effort at every site was consistent because one sample was always taken by each of the first two authors. Most samples were taken from an area of about 0.5 m^2, but if snails/shells were scarce the area covered in 1 h was larger. In addition, 15 min searches for tree snails, looking at leaves, trunks and under bark, and covering 10 trees or shrubs within 30 m of the flag, were conducted at every site sampled. Litter/soil samples from a 30 × 30 cm area were collected at five sites. Because sampling took place during day-time and most snail species are essentially nocturnal, snail distributions necessarily represent resting sites rather than sites of activity. However, given that most of the species are very

small (2–10 mm) and probably do not travel far, the scale of the sampling protocol would be unlikely to allow resting and active sites to be distinguished.

Sorting and Identification of Material

All specimens collected during the field trips were sorted, counted, and identified as far as possible. Litter samples were sifted using standard mesh screens of decreasing mesh size, followed by scanning of all sifted soil and litter. Identifications were made by reference to the extensive malacological collections of the Bishop Museum (Honolulu), and to appropriate literature. Specimens were recorded as "live," "dead recent" (shell with at least half the periostracum still present, and the shell retaining its original color), and "dead old" (less than half the periostracum still present, and/or shell opaque white). Only intact shells or fragments of shells containing the shell apex, and identifible non-apical fragments of species otherwise not represented in a particular sample, were counted (cf. Christensen & Kirch, 1986). This protocol removes the possibility of counting a single shell more than once, but does leave uncounted a number of readily identifiable specimens. Nevertheless, it is deemed a more rigorous approach, did not exclude a large amount of identifiable material, and therefore does not influence the overall conclusions of the study.

Despite extensive previous systematic study of the Hawaiian land snail fauna, there remain many undescribed species. In particular, the island of Hawaii is less well known malacologically than the other islands (Cowie, in press). In addition, the extent of intra-specific variation is often unknown. Therefore, it is frequently difficult to identify material to a recognized species. This is the case in the present study. However, most of the material collected could be assigned to distinct "morphospecies," even though a specific name could not be applied with certainty. All material is deposited in the malacology collections of the Bishop Museum (TL-1994.050).

Environmental Variables

Six environmental variables were recorded at each site. Values of the vegetation-related variables were derived from data accumulated as part of the non-malacological aspects of the overall environmental assessment.

(1) Altitude: Taken from a 1:50,000 map of the Pohakuloa training area with contour intervals of 12.2 m (40 ft).

(2) Canopy height: Scored in the field in 8 classes, from 1 for no canopy, to 8 for canopy height greater than 10 m.

(3) Canopy closure: Scored in the field in 12 classes, from 1 if the site was completely open, to 12 if the canopy was more than 50% closed.

(4) Vegetational community: Four communities were recognized, based on the dominant trees or shrubs (see introduction). They were coded as follows: 1—bare lava or very sparse pioneer vegetation, 2—*Metrosideros* forest, 3—*Sophora/Myoporum* forest, 4—*Dodonaea* shrubland.

(5) Vegetational heterogeneity: A measure of combined canopy and understory heterogeneity, reflecting the overall vegetational heterogeneity of the site, was obtained as follows. For the canopy, a score of 1 was given when one canopy species was dominant and a score of two when two or more species were codominant. The understory was categorized into bare substratum, native shrubs, native grasses, native ferns, native herbs, alien shrubs, alien grasses, alien herbs, alien vines. One or as many as four of these nine elements could be considered dominant or codominant at a particular site, giving a score of 1–4 for increasing understory heterogeneity. By adding the canopy and understory scores, the combined heterogeneity score therefore ranged between 2 and 6.

(6) Lava type: The substratum from which the actual samples were taken, i.e., pahoehoe (coded as 1) or aa (coded as 2) (Fig. 1).

Statistical Analysis

There were only minor differences in the presence/absence of particular species between the two samples taken at each site. However, log-likelihood G statistic analysis (Rohlf & Sokal, 1969; Sokal & Rohlf, 1981) of the 46 sites at which there were sufficient numbers of specimens, indicated highly significant differences in relative abundances between the two samples ($p < 0.001$ in 30 cases, $p < 0.01$ in six cases, $p < 0.05$ in two cases), due perhaps both to different biases between the two people performing the sampling or to differences in microhabitat between the two sample locations. Neverthe-

less, in order to obtain a more general picture of the fauna at each site and to relate the snail distributions to environmental variables at the meso-scale of the sites rather than the micro-scale of the individual samples, the data for the two samples at each site have been combined for the purpose of the following analysis.

The program ADE 3.6 (Chessel & Dolédec, 1993) was used to carry out a canonical correspondence analysis (CCA; Ter Braak, 1986; Lebreton et al., 1988; Palmer, 1993) on the abundance (combined number of live and dead individuals) of each species at each site in relation to the six environmental variables indicated above. CCA is particularly appropriate when species show non-linear relationships with environmental variables (Ter Braak, 1986), as is recognized may often be the case in studies of molluscs (Bishop, 1981). CCA is designed for gradient analyses, that is, analyses of species distributions along environmental gradients. Neither vegetational community nor lava type is a gradient variable. However, they have been incorporated into the CCA as pseudo-gradient variables for exploratory purposes. The four sites at which no snails were found (sites 16, 17, 31, 57) were included in the CCA, but the single site (site "x") from which only one sample was available was excluded because snail abundance at that site would be underestimated. Also, specimens referred to "*Leptachatina* sp." were excluded as being unidentified specimens that probably belonged to the other *Leptachatina* spp. recorded.

TABLE 1. Land snail taxa found during the survey.

ACHATINELLIDAE
Lamellidea sp.
Tornatellides sp(p).
AMASTRIDAE
Leptachatina (*L.*) *lepida* Cooke, 1910
Leptachatina (*Angulidens*) *anceyana* Cooke, 1910
Leptachatina sp. A
Leptachatina sp. B
Leptachatina sp. C
Leptachatina sp.
PUPILLIDAE
Nesopupa (*Infranesopupa*) *subcentralis* Cooke & Pilsbry, 1920
Pronesopupa sp.
SUCCINEIDAE
Succinea konaensis Sykes, 1897
HELICARIONIDAE
Euconulus (*Nesoconulus*) sp. cf. *gaetanoi* (Pilsbry & Vanatta, 1908)
Philonesia sp.
ZONITIDAE
Nesovitrea hawaiiensis (Ancey, 1904)
Striatura (*Pseudohyalina*) sp. cf. *meniscus* (Ancey, 1904)
?*Striatura* sp.
Vitrina tenella Gould, 1846

RESULTS

Taxonomy

At least 16 species are represented in the collections (Table 1). All but one of them are probably endemic to the Hawaiian Islands (*Vitrina tenella* is native but not endemic.) Their classification here follows Cowie et al. (in press). The taxonomy of many of the groups is uncertain; no previous collections have been made in the area of the study, and, with the often highly localized distributions of Hawaiian land snail species, it is probable that a number of the species found are undescribed. Problematic taxa are now briefly discussed.

Lamellidea sp.: Only three species of *Lamellidea* have been recorded from the island of Hawaii: *L. gracilis* (Pease, 1871), *L. oblonga* (Pease, 1865) and *L. peponum* (Gould, 1847). Their shell morphology is similar, but material in the Bishop Museum shows a range of variation both within and among individual lots, including type lots, with some overlap between lots referred to different species. Both *L. gracilis* and *L. oblonga* have been considered lowland species that probably do not reach the altitude of the study area (Cooke & Kondo, 1960), but it is not possible to identify the present material more precisely.

Tornatellides sp(p).: The genus *Tornatellides* can be difficult to distinguish from other closely related genera, particularly *Tornatellaria.* However, *Tornatellides* bears live young, whereas *Tornatellaria* lays eggs (Cooke & Kondo, 1960). The present material manifests some variation, especially in size, but is all tentatively referred to the genus *Tornatellides,* because embryos were found inside some individuals. The variation exhibited perhaps suggests more than one species, although this variation may yet be intraspecific, with only a single, rather variable species being represented. Referral to particular species is not possible.

Leptachatina sp. A: This species, although somewhat variable in size, appears to be distinct. It is rather tall and narrow with a relatively large protoconch. It is somewhat similar to *L. imitatrix* Sykes, 1900, but probably represents an undescribed species.

Leptachatina sp. B: Specimens assigned to this species appear somewhat intermediate between *L. lepida* Cooke, 1910, and *L. konaensis* Sykes, 1900, being fatter than the former but thinner than the latter. They may belong to one of these species, or may represent an undescribed species, but a firm decision depends on future revision of the group.

Leptachatina sp. C: Specimens assigned to this species are similar to but appear distinct from *L. lepida.* They are large, with a rather straight, not convex, outline to the shell spire, and perhaps represent an undescribed species. Further taxonomic research will be required to confirm their true status.

Pronesopupa (*Sericipupa*) sp.: Referral of these specimens to subgenus appears fairly secure. However, they do not correspond precisely to any of the three species—*P. lymaniana* Cooke & Pilsbry, 1920, *P. orycta* Cooke & Pilsbry, 1920, *P. sericata* Cooke and Pilsbry, 1920—described from the island of Hawaii and may represent an undescribed species.

Euconulus (*Nesoconulus*) sp. cf. *gaetanoi:* The present material does not correspond precisely to anything in the Bishop Museum collections but is nevertheless very close to *E. gaetanoi.* It may or may not be a distinct species.

Striatura (*Pseudohyalina*) sp. cf. *meniscus:* Type material of *S. meniscus,* held at the Bishop Museum, contains a range of morphological variation, especially in umbilicus width, and in fact seems to include two species. The holotype has a wide umbilicus, whereas the specimens from the present survey correspond very closely to those paratypes with the narrower umbilicus, which resemble *S. pugetensis* Dall, 1895. Detailed taxonomic work, beyond the scope of this ecological study, is necessary to decide whether the present specimens are indeed *S. meniscus* or *S. pugetensis,* or whether they belong to a further, closely related, but possibly undescribed species. Baker (1941) hinted at this confusion.

?*Striatura* sp.: Specimens from the present survey, distinct from the previous species, nevertheless appear closely related to it conchologically and so are tentatively assigned to the genus *Striatura.* Material of this species in the Bishop Museum collections has been labeled *S. meniscus,* but incorrectly. The survey specimens do not correspond to anything in the type collections of *Striatura* in the Bishop Museum nor to the written treatment of Baker (1941). They cannot be identified further and may belong to an undescribed species.

Philonesia sp.: Although clearly belonging to the genus *Philonesia,* the present material, apparently of one species only, does not correspond closely with material of any of the *Philonesia* spp. from the island of Hawaii held in the Bishop Museum collections. It is possibly an undescribed species.

Overall Abundance

A total of 12,273 specimens (252 live) was collected by hand searching in the field, with an additional 2,342 (563 live) being extracted from the soil/litter samples. No arboreal species were found, although ?*Striatura* sp. was found on tree trunks on one occasion at one site. The raw data are held by the first author and summarized in the appendices of Cowie & Nishida (1993).

The vast majority of specimens collected were empty shells. Only 10 of the 16 taxa were recorded live, and then, except in the case of ?*Striatura* sp., which was the most common species found in the survey, only with very few live individuals. Species richness at a particular site ranged from two to 12 species.

In only one instance did the litter/soil samples detect a taxon not represented in the regular field samples at a particular site (*Tornatellides* sp. found in very low numbers at site 25). In all other cases, the regular samples detected more species than the soil/litter samples. Not unexpectedly, however, of the species recorded from the soil/litter samples, relatively greater numbers of the smaller species were recorded in these samples compared to the regular samples. In one instance (site 45), the soil/litter sample detected a species alive (*Pronesopupa* sp., three live out of a total of 34) that was only represented by dead (dead recent) shells in the regular field samples at that site.

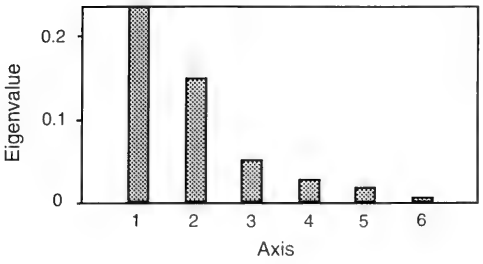

FIG. 2. Partitioning of eigenvalues across the six axes extracted by the CCA.

Lamellidea sp., *Succinea konaensis* and ?*Striatura* sp. were the most abundant (over 1,000 specimens of each), although in the case of *Lamellidea* sp. by no means ubiquitously distributed. The rarest species (less than 100 specimens each) were *Leptachatina anceyana, Leptachatina* sp. B, *Leptachatina* sp. C, *Pronesopupa* sp., *Euconulus* sp. cf. *gaetanoi* and *Vitrina tenella.* The remaining species were intermediate in abundance.

Patterns of Distribution

General Patterns Detected by the CCA: The total eigenvalue for the six axes extracted by the CCA is 0.474, partitioned according to Figure 2. Much of the variance (79%) in species abundance by site, as constrained by the environmental variables incorporated in the CCA, was explained by the first two axes. The ordination diagram (Fig. 3) describes the relations among species and sites, as related to the six environmental variables, on the first two axes of the CCA.

In addition to the CCA, a correspondence analysis (CA) was performed on the abundance (combined number of live and dead individuals) of each species at each site, and the results compared to those of the CCA, as recommended by Ter Braak (1986). The species scores on axis 1 and axis 2 of the CA were not highly correlated with those of the CCA ($r = -0.466$, $p = 0.069$ and $r = -0.039$, $p = 0.887$, respectively, $n = 16$). The correlations for axes 3 and 4 were better ($r = -0.488$, $p = 0.055$ and $r = 0.651$, $p = 0.006$, respectively), but these axes only explained a small additional amount of the overall variance (Fig. 2). This poor correlation for axes 1 and 2 weakens the robustness of the CCA, which must therefore be evaluated cautiously. Following Borcard et al. (1992), it is possible to estimate the proportion of the total variance explained by the environmental variables by dividing the total eigenvalue of the CCA (0.474) by that of the CA (1.944). This indicates that 24% of the overall variance in species abundances is explained by the current environmental variables and that 79% of this is explained by axes 1 and 2 of the CCA.

Interpretation of the ordination diagrams generated by a CCA is clearly explained by Ter Braak (1986). The length and direction of an arrow representing an environmental variable indicates the importance of the variable in the formation of the axes: the smaller the angle between the arrow for a particular variable and an axis, the larger the contribution of the variable to that axis; the longer the arrow relative to other arrows, the greater the contribution. The score of a species or site on a particular environmental variable is determined by dropping a perpendicular from the species or site point to the arrow (or to the imagined extension of the arrow) representing that variable. A high score (positive or negative) on the arrow represents a strong association of the species or site with that variable.

In the present case, axis 1 is closely related to lava type, and lava type is the most important variable among those included in the study (Fig. 3, Table 2). Both the canonical coefficients and intraset correlation coefficients (Table 2) for lava type are distinctly greater than those for any other variable. In the ordination diagram (Fig. 3), all sites to the left of the altitude and canopy height arrows are on aa lava and all to the right are on pahoehoe.

Axis 2 is more difficult to interpret but appears most closely related to altitude (Table 2). Inspection of the ordination diagram (Fig. 3) reveals indeed that those sites falling in the upper part of the diagram are high altitude sites whereas those in the lower part are low altitude sites.

The ordination diagrams for axes 3 and 4 are not presented, because these axes make only minor contributions to explaining the overall variance. They are difficult to interpret because neither is clearly related to just a single variable, although axis 3 may be a composite of the vegetational variables.

Inter-specific Associations and Overall Strength of Environmental Associations: The ordination diagram (Fig. 3) indicates no clear clusters of species, suggesting that there are

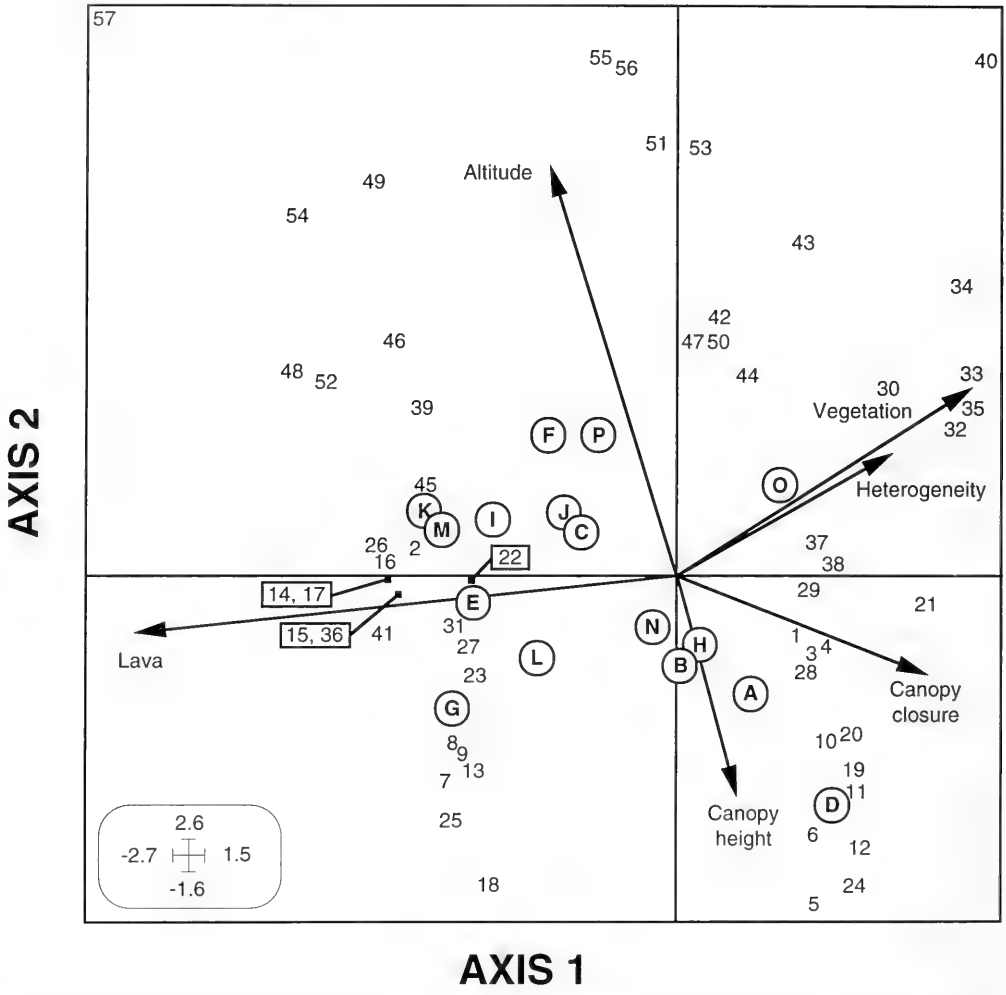

FIG. 3. Ordination diagram of site and species distributions on axes 1 and 2 of the CCA. Sites are indicated by the numbers as given in Figure 1. Snail species are represented as follows: A—*Lamellidea* sp., B—*Tornatellides* sp(p)., C—*Leptachatina lepida,* D—*Leptachatina anceyana,* E—*Leptachatina* sp. A, F—*Leptachatina* sp. B, G—*Leptachatina* sp. C, H—*Nesopupa subcentralis,* I—*Pronesopupa* sp., J—*Succinea konaensis,* K—*Euconulus* sp. cf. *gaetanoi,* L—*Philonesia* sp., M—*Nesovitrea hawaiiensis,* N—*Striatura* sp. cf. *meniscus,* O—?*Striatura* sp., P—*Vitrina tenella.* The arrows representing the environmental variables are scaled up by a factor of 6.49 for clarity of presentation (see Ter Braak, 1986).

no strong associations among snail species, that is, no clear sub-communities. The species all plot rather close to the center of the ordination diagram (Fig. 3), also indicating that none of them has a particularly strong association with any of the environmental variables incorporated in the analysis. Associations with the most significant variables are presented below.

Local Rarity and Patchiness: Certain species were found only in very low numbers and/or at very few sites. This rarity may be only a local phenomenon and not reflect overall rarity on the island of Hawaii. Some apparent patterning in the distributions of these rare species may simply be due to sampling error. For instance, *Pronesopupa* sp. was recorded in very low numbers (< 10 at any one site)

TABLE 2. Canonical coefficients and intraset correlation coefficients (see Ter Braak, 1986) of the environmental variables with the first four axes of the CCA.

Variable	Canonical coefficients				Correlation coefficients			
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 1	Axis 2	Axis 3	Axis 4
Altitude	−0.33	0.54	0.02	0.80	−0.22	0.82	0.09	0.56
Canopy height	0.07	−0.52	1.12	0.43	0.10	−0.44	0.63	0.01
Canopy closure	−0.08	−0.54	−0.16	−0.06	0.42	−0.20	0.33	−0.41
Vegetational community	0.38	0.15	1.33	−0.01	0.50	0.38	0.53	−0.34
Vegetational heterogeneity	−0.04	0.47	−0.43	−1.02	0.37	0.24	0.54	−0.43
Lava type	−0.87	−0.09	0.35	−0.48	−0.92	−0.12	−0.03	−0.20

from only four sites in the higher parts of the main study area (sites 41, 45, 52, 56) and from only three along the approach road (2, 9, 10). Of these seven sites, six were on aa lava. This apparently disjunct distribution, and the high score on the lava type arrow may therefore simply be sampling artifacts.

The high scores of certain species on the altitude arrow are possibly reflections of their rarity. For instance, *Leptachatina anceyana* was only found, in low numbers, at five sites towards the lower part of the main study area (sites 24, 29, 30, 32, 37). *Leptachatina* sp. B was recorded from only six sites (15, 24, 43–46), although four of these were on a single transect (M5) and might reflect real patchiness. *Euconulus* sp. cf. *gaetanoi* was recorded at only two sites (27, 45) and the relatively high score on the altitude arrow probably reflects its greater abundance at the higher of these sites. *Vitrina tenella* was also collected at only two sites (44, 45). However, these were close together on a single transect (M5); both were on pahoehoe lava and in *Sophora/Myoporum* forest. If this species were widely but sparsely distributed over the whole study area, one would not have expected the two collection localities to be so close together, perhaps suggesting that this is indeed a very localized distribution.

The absence of rare species from certain vegetational communities (Table 3) may well also be a sampling artefact related not only to the overall rarity of the snail species but also to the different numbers of sites of each community type that were sampled. Only *Vitrina tenella* is confined to a single vegetational community, but this may be an artefact of its extreme rarity in the study area. Absence of *Leptachatina* sp. C, *Pronesopupa* sp. and *Euconulus* sp. cf. *gaetanoi* from communities characterized as *Dodonaea* shrubland and as bare lava or very sparse pioneer vegetation may be a reflection of both the overall rarity of these species and the relatively few sites of these communities that were sampled. Absence of *Leptachatina* sp. B from *Dodonaea* shrubland and *Leptachatina anceyana* from bare lava or very sparse pioneer vegetation may be explained in a similar way. However, the absence of the more common *Leptachatina* sp. A and *Nesovitrea hawaiiensis* from *Dodonaea* shrubland may reflect a real phenomenon (see below).

TABLE 3. Presence (+) or absence (0) of land snail taxa with vegetational community, coded as follows: 1—bare lava or sparse pioneer vegetation; 2—*Metrosideros* forest; 3—*Sophora/Myoporum* forest; 4—*Dodonaea* shrubland.

Land snail taxa	Vegetational community			
	1	2	3	4
Lamellidea sp.	+	+	+	+
Tornatellides sp(p).	+	+	+	+
Leptachatina lepida	+	+	+	+
Leptachatina anceyana	0	+	+	+
Leptachatina sp. A	+	+	+	0
Leptachatina sp. B	+	+	+	0
Leptachatina sp. C	0	+	+	0
Leptachatina sp.	+	+	+	+
Nesopupa subcentralis	+	+	+	+
Pronesopupa sp.	0	+	+	0
Succinea konaensis	+	+	+	+
Euconulus sp. cf. *gaetanoi*	0	+	0	0
Philonesia sp.	+	+	+	+
Nesovitrea hawaiiensis	+	+	+	0
Striatura sp. cf. *meniscus*	+	+	+	+
?*Striatura* sp.	+	+	+	+
Vitrina tenella	0	0	+	0

Lava Type: All but two species were found on both lava types. *Euconulus* sp. cf. *gaetanoi* was only recorded from aa and *Leptachatina anceyana* only from pahoehoe. Both these species score highly on axis 1 of the CCA (Fig. 3), which appears closely related to

lava type, but both are so rare (only recorded at two and five sites, respectively) that this apparent association may be due to chance and of no real biological meaning. Other species scoring highly on axis 1 are *Leptachatina* sp. A, *Leptachatina* sp. C, *Pronesopupa* sp., *Philonesia* sp., *Nesovitrea hawaiiensis* and ?*Striatura* sp. The distributions of *Leptachatina* sp. A, *Philonesia* sp. and *Nesovitrea hawaiiensis* are somewhat similar to each other and show similar associations with lava type, as follows. Sixteen of the 21 sites at which *Leptachatina* sp. A was found, 17 of the 23 at which *Philonesia* sp. was found, and 19 of the 26 at which *N. hawaiiensis* was found, were on aa. All these associations are statistically significant (G tests; $p < 0.005$ in all cases). *Leptachatina* sp. C and *Pronesopupa* sp. were found at five sites (four of them aa) and seven sites (six of them aa), respectively, but this is too few for G statistic analysis. All these species that appear to show an association with aa plot on the ordination diagram (Fig. 3) among the left hand cluster of sites, all of which are on aa. Two species, *Leptachatina anceyana* and ?*Striatura* sp., plot among the pahoehoe sites on the ordination diagram. Of these two species, *L. anceyana* occurred at very few sites (see above), but ?*Striatura* sp. was both widespread and abundant, and, although in terms of presence/absence at aa or pahoehoe sites it showed no significant association, it occurred in significantly greater numbers on pahoehoe (G test: $p < 0.001$).

Altitude: Taxa scoring highly on the altitude arrow (Fig. 3) are *Lamellidea* sp., *Leptachatina anceyana, Leptachatina* sp. B, *Euconulus* cp. cf, *gaetanoi* and *Vitrina tenella.* Of these, only *Lamellidea* sp. is at all common, being overall the second most abundant species recorded. It is completely restricted to the lower parts of the study area (all approach road transects and transects M1–3). The high scores of the other species are possibly reflections of their rarity.

Vegetation: The presence/absence of snail species, according to the vegetational community at each site are presented in Table 3. The CCA indicated no strong influence of any of the vegetational variables on snail species distributions, although the ordination diagram (Fig. 3) clearly grouped the sites characterized as *Dodonaea* shrubland to the far right and those as bare lava or very sparse pioneer vegetation to the left, with the other two vegetation types scattered between them. None of the snail species appears particularly associated with any vegetational community, nor with any other vegetational characteristic (but see below).

Nevertheless, direct inspection of the data indicates that *Leptachatina* sp. A, *Philonesia* sp. and *Nesovitrea hawaiiensis* have somewhat similar distributions, being absent or nearly absent from the central and more northerly sites on transects M2–4. This gap in the distributions of these three species appears roughly to correlate with the presence of *Leptachatina anceyana* (only recorded in the lower part of the main study area, at a total of five sites on transects M1–4) and with a concentration of *Dodonaea* shrubland along the more northern end of transect M3. (Casual observations suggested that this part of the study area also supported the highest concentration of feral sheep, which may have had an impact on both physical and chemical soil characteristics.) These patterns perhaps suggest some ecological interaction or differential habitat preferences between the snail species. *Leptachatina anceyana* plots to the far right of the ordination diagram (Fig. 3), as do *Dodonaea* shrubland sites.

Leptachatina sp. A, *Philonesia* sp. and *Nesovitrea hawaiiensis,* as well as perhaps having a negative association with *Dodonaea* shrubland, are all positively associated with aa lava. In addition, all five sites at which *Leptachatina anceyana* was recorded were on pahoehoe. Because the CCA indicated a much greater influence of lava type, the relationship with lava type may be more important than the apparent relationship with vegetational community for these species.

DISCUSSION

Although the land snail fauna of the Hawaiian islands is one of the most species-rich in the world for an area of comparable size (cf. Solem, 1984), the local species richness recorded in the present study is not exceptional. Only 16 species were found, with no more than 12 at any one collection site. While truly valid comparisons of species richness can only be made in terms of area (and sampling effort), this local species richness seems comparable to that found in similar sampling programs in many other parts of the world, that is, ranging up to about 30 species

but usually fewer (e.g., Bishop, 1981; Solem, 1984; Cameron, 1992). Greater numbers might be found in particularly diverse areas, but the species might not be truly sympatric on a small scale. The most notable exception is the finding of over 70 species that were indeed suggested as being "microsympatric" in small patches of forest in New Zealand (Solem et al., 1981; Solem, 1984; Solem & Climo, 1985); and a few other regions (in the Caribbean and Australia) are also known to support high levels of microsympatric snail species (Solem, 1984). The relatively low numbers in the present survey, combined with the extraordinary species richness in the Hawaiian islands as a whole (Cowie et al., in press), reflect the fact that most Hawaiian land snail species are highly localized either geographically (i.e., particular parts of an island, a valley, ridge, etc.) or ecologically (i.e., lowland, rainforest, etc.). Additionally therefore, because collections have not previously been made in the area of the study, it is not surprising that much of the present material appears to represent undescribed species.

The comparison of the total eigenvalues of the CA and the CCA indicates that the environmental variables incorporated in the study explained 24% of the overall variance in species abundance by site. The remaining variance may be partly explained by other abiotic and biotic factors, as well as by stochastic variation, especially related to historical factors (cf. Bishop, 1981). Such abiotic factors as pH, calcium availability and soil humidity, are known to influence snail distributions elsewhere, although their effects are not always straightforward (Cameron, 1978; Peake, 1978; Bishop, 1981; Cain, 1983). Unfortunately, it was not possible to obtain appropriate data to incorporate these variables into the present study. Such biotic factors as competition and predation, have only rarely been demonstrated as influencing the spatial distributions or abundances of land snails (Mordan, 1977; Peake, 1978; Cain, 1983; Cowie & Jones, 1987; Smallridge & Kirby, 1988). Historical factors have been shown to be important (Cameron & Dillon, 1984), but very few studies have addressed this question. It is not possible to speculate on the relative importance of these factors in relation to the unexplained variance in the present study. It is not uncommon in studies of this kind for a relatively large part of the variance to remain unexplained; but the factors that are found to have significant influences may yet be important in structuring the community under study (Borcard et al., 1992). Nevertheless, it is also possible that the distributions of the species are heavily influenced by environmental variability on a much finer micro-scale than incorporated in the present analysis. This is suggested by the highly significant differences in relative abundances of species between the two samples taken at each site.

Of the 24% of the overall variance at the meso-scale explained by the factors included in the study, 79% is explained by the first two axes of the CCA. These two axes appeared to be related most closely to lava type and altitude.

A number of associations of certain species with lava type are clear. However, it is not clear exactly what is the real variable, associated with lava type, that is influencing these associations. The physical characteristics of the two types of lava are very different and may be important for the snails. The smooth surfaces of pahoehoe provide little microhabitat for snails, and in areas of pahoehoe most snails/shells were found in places where there was shade, moisture and an accumulation of litter and soil, such as in the cracks in the lava or at the bases of slabs of lava. The broken nature of aa lava provides much greater possibilities for shade, but often any soil and litter was found only deep down after removing many chunks of lava. It could be argued that pahoehoe is more variable physically. Young pahoehoe is smooth, but as it ages it can break down into small rocks and boulders that perhaps offer habitats more akin to aa. This greater physical variability may be reflected in the wider spread of pahoehoe sites than of aa sites on axis 1 of the CCA (Fig. 3). There is no significant difference in chemical composition between the two lava types (Peterson & Tilling, 1980; Vitousek et al., 1992), so soil pH is not influenced directly by lava type. However, the vegetation supported on the two types of lava may differ (Karpa & Vitousek, 1994), as was suggested by the CCA. Different vegetation might influence such factors as soil chemistry, and depth and decomposition rate of litter, but the vegetation-related variables incorporated in the study were not strongly related to the snail faunal composition. Vitousek et al. (1992), whose study area encompassed that of the present study, found differences among sites in accumulation of carbon, nitrogen and phosphorus in soils,

availability of soil nutrients, and in foliar nutrients of *Metrosideros polymorpha,* the dominant tree of their study. Although some of these differences were related to altitude, lava type and flow age, there seemed to be no consistent pattern, and our understanding of variations in these factors on the dry, northwest slopes of Mauna Loa remains poor (Vitousek et al., 1992). Karpa & Vitousek (1994) hinted at other possible differences between the lava types (local flooding and susceptibility of the vegetation to fire), but it seems unjustified to speculate further on the importance of these rather poorly understood environmental variables in influencing the distributions of the snail species in the present study.

Only one species, *Lamellidea* sp., is sufficiently abundant to suggest reliably that its recorded distribution relates to altitude. It is possible that the study area located the upper altitudinal boundary of this species, since, although it could not be decisively identified, at least two of the three species of *Lamellidea* previously recorded from the island of Hawaii are lowland taxa (Cooke & Kondo, 1960). This clear relation of the distribution of *Lamellidea* sp. to altitude and the more tentative overall association of the faunal composition with altitude may be related to such factors as temperature and rainfall. Cameron (1978) indicated decreasing land snail species richness at higher altitudes in his study area in England and implied a relationship with local climate. Certainly climatic factors have frequently been considered of fundamental importance in determining snail distributions (e.g., Peake, 1978; Arad, 1990; Asami, 1993; Baur & Baur, 1993). There is a gradient, at least in temperature and perhaps in rainfall, related to altitude in the study site, although the range is small (Armstrong, 1983; Giambelluca et al., 1986), and variation on a microhabitat scale might be more important. But, in the absence of appropriate data on temperature tolerance, resistance to desiccation, and other factors, of the snail species (cf. Baur & Baur, 1993), further speculation is not justified.

The lack of a clear relationship with vegetational community or any other vegetational variable, except for the extremely tentative associations (both positive and negative) of some species with *Dodonaea* shrubland, is a little surprising. This lack of relationship suggests that the significant differences between the two samples at each site might be related to environmental heterogeneity on a much smaller, micro-scale.

The land snail fauna of the Hawaiian islands is recognized as being under serious threat of extinction, with many species already gone (Hadfield, 1986; Solem, 1990). The vast majority of specimens collected were empty shells. The recording of empty shells as "dead old" and "dead recent" was done in an attempt to get some feel for the likely recent and perhaps continuing presence of species that were not recorded live (six out of 16). However, it is not known how long it takes for shells to lose their periostracum and to turn white and opaque. It may well be a matter of years rather than weeks or months, and will probably differ among taxa and among localities according to such things as exposure to sunlight, rainfall and soil acidity. However, the fact that all species, even if not collected alive, were recorded as "dead recent" at at least one site suggests that all the species recorded in the study area are probably still extant. Relative rarity in the study area may serve as an explanation of the absence of live individuals for *Vitrina tenella* (only 2 specimens found), *Leptachatina* sp. B (14 specimens), *Euconulus* sp. cf. *gaetanoi* (25), and perhaps for *Leptachatina anceyana* (46) and *Leptachatina* sp. C (69). *Leptachatina* sp. A was also not found alive but occurred in somewhat higher abundance (234 specimens in the field samples), although it could not be considered common. Species found in abundance but only, or almost only, as dead shells may be extinct or closer to extinction than rare species that were nevertheless found alive, or indeed than common species found in high numbers both dead and alive. For instance, ?*Striatura* sp. is the most abundant species in terms of both live snails and dead shells, but *Lamellidea* sp., the second most abundant in terms of dead shells, was among the rarer species in terms of numbers of live snails found (eighth out of the ten species collected alive). This high relative number of *Lamellidea* sp. shells might reflect a recent increase in the mortality of this species and the possibility that it may become locally extinct in the near future. But it might also reflect the possibility that *Lamellidea* sp. shells do not disintegrate as rapidly as those of ?*Striatura* sp. Such possibilities can only be extremely speculative because nothing is known of such factors as relative differences in rates of shell weathering and breakdown among different species, differences in life histories and rates of mortality.

GENERAL CONCLUSIONS

The land snail community of the study area is composed mostly of species endemic to the island of Hawaii. Significant relationships between their distribution within the study area and at least two environmental variables (lava type and altitude) have been demonstrated. Identification of survey material by reference to the Bishop Museum collections (there are no previous collections specifically from the survey area) and assessments of their previously known distributions suggest that the species recorded, while not unique to the study area, are representative of a fauna characteristic of the Kona side of the island of Hawaii and perhaps more specifically to the Hualalai-Puuwaawaa area. However, much of the material in the Museum was collected many years ago. Nothing is known of the current status of the species at those earlier collecting localities. With the increasing impacts of alien plants and animals introduced to the Hawaiian Islands (Cowie, 1992), it is quite possible that these snail species have declined or gone extinct in these localities. The present survey area, especially for those species recorded alive, is therefore an important part of their known distribution. It is the only area in the Hawaiian Islands for which such a detailed faunistic survey of land snails has been carried out.

The finding of living *Leptachatina lepida* is particularly noteworthy because the study area is the only known locality at which this species is known to be still extant. *Leptachatina lepida* is only one of as few as perhaps six or ten extant species of the Hawaiian endemic family Amastridae, which once numbered over 400 species-group taxa (Cowie et al., 1994). Amastrids, and perhaps the genus *Leptachatina* in particular, seem highly susceptible to habitat modification (Christensen & Kirch, 1986). The survey area is therefore of particular significance in the preservation of what remains of this unique and once highly diverse family.

The survey was conducted as part of an assessment of the potential environmental impact of military activities on the fauna. Military use of the study area has had and will continue to have direct impacts on the snails (e.g., explosions, construction work). Furthermore, and perhaps most significantly, it will probably result in habitat change that is likely to be highly detrimental to the fauna. In addition, other factors, such as grazing by introduced feral ungulates (Cameron, 1978), changes in the floral composition of the area due to the increasing numbers of introduced species (Karpa & Vitousek, 1994), and predation by rodents (Hadfield et al., 1993), ants (Solem, 1976) and perhaps introduced gallinaceous birds (Buckle, 1989), are all serious threats to this unique community.

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GENETIC HETEROZYGOSITY AND GROWTH RATE IN THE SOUTHERN APPALACHIAN LAND SNAIL *MESODON NORMALIS* (PILSBRY 1900): THE EFFECTS OF LABORATORY STRESS

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ABSTRACT

Mesodon normalis hatchlings (totaling 569) exposed to a stress involving excess moisture, mucus, and feces exhibited a reduced mean growth rate, an increased mortality rate, and a significant positive association between juvenile growth and genetic heterozygosity. No significant genotype-dependent growth and mortality occurred in an unstressed control cohort of 458 offspring. The significant r^2 value 0.053 in the stressed cohort is consistent with values from comparable studies in marine bivalves. Two of the five enzyme loci (ALA and PGI) contributed significantly to the association in the stressed cohort, but neither heterozygous deficiencies nor level of heterozygosity were associated with the growth-heterozygosity correlation. When the growth-heterozygosity association was examined in each of the eight broods comprising the stressed cohort, only one clutch from one parent showed a significant growth-heterozygosity association. In six of the remaining seven broods in the stressed cohort and in five of the eight broods in the unstressed cohort, the trend was in the direction of enhanced growth of heterozygotes over homozygotes or failure of the homozygote class to survive.

By comparing the genotypic structure of the parent to its offspring, it was determined that selfing did not occur and that multiple paternity was common during reproduction in this sample of the monoecious *Mesodon normalis*.

These findings have significance for previous work on the population biology of this species in that genotype-dependent growth and survivorship appear to influence more the timing of adulthood and reproduction rather the usual body size-dependent reproductive output reported for many European helicids.

Key words: growth rate, survival, genetic heterozygosity, stress.

INTRODUCTION

One consistent finding from research on the regulation of helicid land snail abundance is an inverse association between population density and such fitness components as juvenile growth rate and survivorship, and adult body size (e.g. Dan & Bailey, 1982; Baur, 1988; Perry & Arthur, 1991). However, the relationship of reproductive output to growth, body size and adult age is less well established (e.g. Carter & Ashdown, 1984; Baur, 1988; Baur & Baur, 1990; Foster & Stiven, in press, in review). Considerable research exists on genetic variation in land gastropod populations (e.g. the European *Cepaea* spp.) (Cain, 1983), and thus any investigation of mechanisms controlling the growth, body size, and age connections cannot ignore the possible importance of a genotype and growth rate association. This study focuses on the importance of laboratory induced stress in the southern Appalachian land snail *Mesodon normalis* on this association.

Positive associations between shell size or growth rate and genetic heterozygosity are common among mollusks (Zouros & Foltz, 1987). This association often appears when loci show heterozygote deficiencies, when the sample comes from a natural rather than a laboratory population (Zouros, 1987), when the sample consists of a random population sample rather than offspring of a single mating (Mallet et al., 1986), when the sample consists of a young rather than an older cohort (Diehl & Koehn, 1985), or when the number of loci examined is large (Koehn et al., 1988). Explanations range from overdominance (Zouros et al., 1980, 1983), to the presence of null alleles (Foltz, 1986), and to different responses of genotypes to environmental stress (Koehn & Shumway, 1982; Hawkins et al. 1986; Holley & Foltz, 1987). Attempts have also been made to assess the contribution of individual loci to

the growth rate-heterozygosity relationship by comparing fitness components between homozygotes and heterozygotes for individual loci, with glycolytic and protein catabolism enzyme loci emerging as being most significant (Koehn et al., 1988; Gentili & Beaumont, 1988; Borsa et al., 1992).

Even though stress was predicted to enhance the heterozygous advantage (Koehn & Shumway, 1982; Mitton & Grant, 1984), allozyme heterozygosity and fitness have been inadequately studied under stressful and optimum conditions (Hoffmann & Parsons, 1991). Most evidence supporting the importance of stress has come largely from bivalves (e.g. Diehl & Koehn, 1985; Gentili & Beaumont, 1988 [but see Gaffney's 1990 reanalysis], Koehn & Bayne, 1989; Scott & Koehn, 1990; Borsa et al., 1992). In gastropods, evidence for the significance of stress in enhancing the relationship between genetic heterozygosity and fitness is scarce (Komai & Emura, 1955; Booth et al., 1990). There are a few studies of aquatic and marine snails in which no association was found in either the stress or control treatment (Fevolden & Garner, 1987; Foltz et al., 1993), or the association persisted over a range of environments in which some could be labeled stressful (e.g. low to high salinity levels; Garton, 1984).

Heritability of shell size in land snails can range from 50% to 70% (Goodfriend, 1986). For growth rate, a major determinant of adult size in land snails, heritability can range from 40% to 60% in *Cepaea nemoralis* (Oosterhoff, 1977). Emberton (in press) also reported a heritability component of 30% and an environmental component of 50% for laboratory growth rate in the southern Appalachian land snail *Mesodon normalis*.

Mesodon normalis (Pilsbry, 1900), which was formerly considered a subspecies of *Mesodon andrewsae* (see Pilsbry, 1940) (Stylommatophora: Polygryidae), is one of the larger endemic land snails of the deciduous forests of the southern Appalachian Mountains of the U.S.A. (Hubricht, 1985). Adult population densities are generally low (< 2/10 m^2) (Foster & Stiven, in review), especially when compared to the European helicid *Cepaea nemoralis,* for which densities range from 5–40/10 m^2 (Perry & Arthur, 1991). *Mesodon normalis* is active on the forest floor, predominately at dawn and dusk (Asami, 1993) from about late April to mid-October. Monoecious adults mate during the spring and early summer, then lay several clutches of up to 110 eggs in the leaf litter. Young emerge from June through August. This species is a determinant grower with a recurved shell lip forming as shell growth ceases and adulthood occurs. Juvenile snails require approximately two years to reach an adult size (27 to 36 mm diameter), and they often continue to live three or more years as adults (Foster & Stiven, in review).

Prior work on *Mesodon normalis* has shown that juveniles reared at lower densities grew faster and became larger adults than did snails reared at higher densities. Survivorship was density-dependent, and the slower growing juveniles had a higher probability of dying younger than their faster growing counterparts (Foster & Stiven, in review). This study examines the association between juvenile growth rate and genetic heterozygosity in clutches of stressed (a period during juvenile growth in the laboratory of increased moisture, feces, and mucus) and unstressed cohorts of *Mesodon normalis*. In particular, this investigation tests the hypothesis that stress (defined as an "environmental change that causes some response by the population" (Underwood, 1989)) causes a genotype-dependent response(s) in an environmentally stressed population.

Parent and offspring genetic data also permitted an assessment of mating patterns (self or cross fertilization, single or multiple paternity) (Anderson & McCracken, 1986; Gaffney & McGee, 1992). For example, Foltz et al. (1984) predicted that successful gastropod colonizers of North American by European species would be self-fertilizing, and that most endemic North American forms found in relatively undisturbed habitats would be outcrossing. In addition, data on shell and tissue color patterns in the offspring are presented as possible markers in future population research.

METHODS

Collections and Laboratory Techniques

To obtain egg clutches for the "stressed" and "unstressed" cohorts, adult *Mesodon normalis* were collected from sites near Highlands, North Carolina, and the U.S. Forest Service Coweeta Hydrologic Laboratory and Experimental Forest (about 25 km east of Highlands) in late May of 1988 and 1989.

Specifically, Highlands snails came from a site (Chin Site) along Highway 106 about 6 km southwest of Highlands (1,170 m elevation), and Coweeta snails came from Watershed 28 (1,100 m elevation) (Stiven 1989; Foster & Stiven, in review, respectively, have more detailed descriptions of these sites).

Adult snails were placed individually in partially covered plastic cylindrical containers (15 cm, diameter, 6 cm deep) which contained a 1 cm layer of soil covered by about 2 cm of leaf litter from the respective sites. Snails were fed daily rations of lettuce dusted with calcium carbonate, and the soil and leaf litter were changed weekly (Cowie & Cain, 1983). Egg clutches were removed immediately after laying and placed into plastic 9 cm petri dishes lined with moist paper toweling. Only first clutches were used in the experiment. After hatching, each of the experimental clutches was placed into 15 cm diameter partially covered plastic chambers lined with moist paper toweling and covered with a 0.5 cm layer of soil and a thin layer of fragmented leaf litter. Snails were fed as above. Chambers were cleaned and soil and leaf litter renewed weekly, except in the stress treatment described below. After one month of snail growth, the chamber was replaced by one of 20.3 cm diameter, and after two months by one of 25.3 cm diameter. All snails that died were removed. The experiment was terminated after 100–115 days of growth. Laboratory temperature ranged from 21°C to 24°C, and the light-dark regime approximated natural conditions.

Experimental Design and Response Variables

The "stressed" and "unstressed" juvenile cohorts were established as follows. Of the 24 adults collected in 1988, eight produced first clutches, six from Coweeta adults and two from Highlands adults. These clutches were the "stressed" cohort and were exposed to a 2-week "stress" period in mid-July, in which chambers were not cleaned and soil and leaf litter were not replaced. Feeding was continued during this period and excess feces, mucus, and moisture was apparent. Individual growth rates were slowed and significant mortality occurred in all chambers of the "stressed" treatment. From the 15 adults collected in 1989, seven first clutches came from Coweeta and one from Highlands snails. These clutches were not stressed during the juvenile period and were the control or "unstressed" cohort. The remainder of the adults failed to produce clutches.

The shell diameters of newly hatched snails of each clutch were measured with a calibrated reticle on a dissecting microscope, and a mean diameter for each clutch was calculated. At the end of the experiment, the shells of surviving individuals were measured with dial calipers to the nearest 0.1 mm, their soft tissue processed for electrophoresis, and shell fragments saved. Growth was exponential during the first 100 days of growth, and the instantaneous growth rate ([ln final size − ln initial size]/days of growth) was calculated for each surviving individual utilizing the mean shell size for each clutch at hatching and its final size and standardizing the growth rate to 100 days of growth. In the stressed cohort, tissue color was noted at time of sacrifice. For the unstressed survivors, tissue color, tissue mottling and shell color were recorded. Tissue colors were light tan, dark brown, and intermediate. Shell colors were either brown or grayish brown, and head tissue mottling or spotting was either present or absent.

Genetic Analysis

Genetic data for the experimental young and adults came from starch gel electrophoresis, as described in Emberton (1988) and Stiven (1989). The entire tissue of young was processed, but the genotype of the adults was assessed by cutting off a small piece of the foot muscle and washing in distilled water before processing. Five polymorphic loci (PGM-2, PGI, MPI, ALA-2, and ALG) were used for the 1988 surviving experimental young. An additional PGM locus (PGM-1) was resolved in the 1989 young. There were two visible loci for the peptidase-leucyl alanine gels but only one (ALA-2) was clear enough to use. All loci except MPI were run on a LiOH buffer. MPI was run on a TEB9/8 buffer. The genotypic and allelic data for the adult group and for each experimental cohort were summarized using Swofford & Selander's (1989) BIOSYS-I program. Measures of genetic heterozygosity included the number of heterozygote loci per individual, mean heterozygosity as direct count and Nei's (1978) unbiased estimate. Departures of genotypic frequencies from Hardy-Weinberg expectations were tested by chi-square with one de-

TABLE 1. Comparisons of clutch size, hatching success, survival, and instantaneous growth rate over the first 100 days of growth for first clutches produced by adult snails under the "unstressed" and "stressed" treatments. Values are means ± SE per clutch (data for eight clutches/treatment).

	Unstressed	Stressed	t	P
Clutch Size (no.)	62.88 ± 4.13	71.75 ± 7.60	1.83	0.09
Hatching Success	0.91 ± 0.02	0.91 ± 0.02	0.15	0.88
Survival	0.81 ± 0.02	0.34 ± 0.05	8.79	<0.0001
Growth Rate (100 days)	0.68 ± 0.03	0.41 ± 0.03	5.74	0.0001

gree of freedom using a pooling procedure giving three genotype classes, homozygotes for the most common allele, heterozygotes for the most common allele and one of the other alleles, and all other genotypes. Heterozygote deficiencies were expressed as $D = (H_o - H_e) / H_e$, where H_o and H_e were observed and Hardy-Weinberg expected heterozygosities respectively. To examine genetic differentiation of the adults between the two sites, F-statistics (Nei, 1977) were also computed for the adult genetic data and significance of F_{ST} tested by chi-square.

Statistical Procedures

The association between multilocus genetic heterozygosity and growth rate was assessed by regressing the instantaneous growth rate (for the 100 days) with the number of heterozygous loci for an individual in each of the "stressed" and "unstressed" cohorts. The association was then examined within individual clutches (family effects) and between the two sites of origin of parents. The relative contribution and significance of each locus to the fitness-heterozygosity association was determined by a multiple linear regression model of growth rate as a function of heterozygosity of each locus as an independent effect (scoring of the genetic state of each locus for each animal as homozygous or heterozygous) following Koehn et al. (1988). Significance of these effects for each locus was assessed by F-tests. The effect of site of origin of the parent, parent-offspring genetic similarity, and tissue or shell color-specific differences utilized either the chi-square test (or G-test), analysis of variance (ANOVA), or analysis of covariance (ANCOVA) when appropriate. Bartlett's test was used to assess homogeneity of variances. All statistical analyses were done using SYSTAT (Wilkerson, 1990). The significance level was $P = 0.05$.

RESULTS

Treatment Conditions

Young hatched after 13–20 days, and eggs of any one clutch hatched within 24 hours of each other. For the 1988 snails, the first clutches used in the experiment appeared between June 14 and June 17, and hatching occurred between June 27 and July 5. For 1989 snails, the comparable dates were May 24 through May 29 and June 9 through June 15. Second and third clutches were produced by some snails into early August. The conditions associated with the treatments (stressed and unstressed) produced the expected differences in levels of survival and growth rate (Table 1). Of the 569 initial number of hatchlings in the eight clutches in the 1988 "stressed cohort," only 192 or 34% survived to the end of the experiment. The comparable figures for the 1989 "unstressed cohort" were 458 hatching from eight clutches, with 373 or 81% surviving. In addition, the growth rate of the survivors in the unstressed treatment was about 65% greater than in the stressed treatment. Clutch size (number per clutch) and hatching success did not differ between the treatments. Adults from the Highland's site did have a significantly greater mean clutch size than those from the Coweeta site (95.0 vs. 65.2, $t_{df=14} = 3.15$, $P = 0.007$), but hatching success, growth rate, and survival did not differ between sites.

Genetic Structure of Adults

The 1988–89 field collection of adults yielded 26 from Coweeta and 13 from Highlands. The level of heterozygosity (direct count) in Coweeta snails was over twice that of Highlands snails based upon five loci (0.292 and 0.129 respectively). All loci, with one exception, had genotypic frequencies

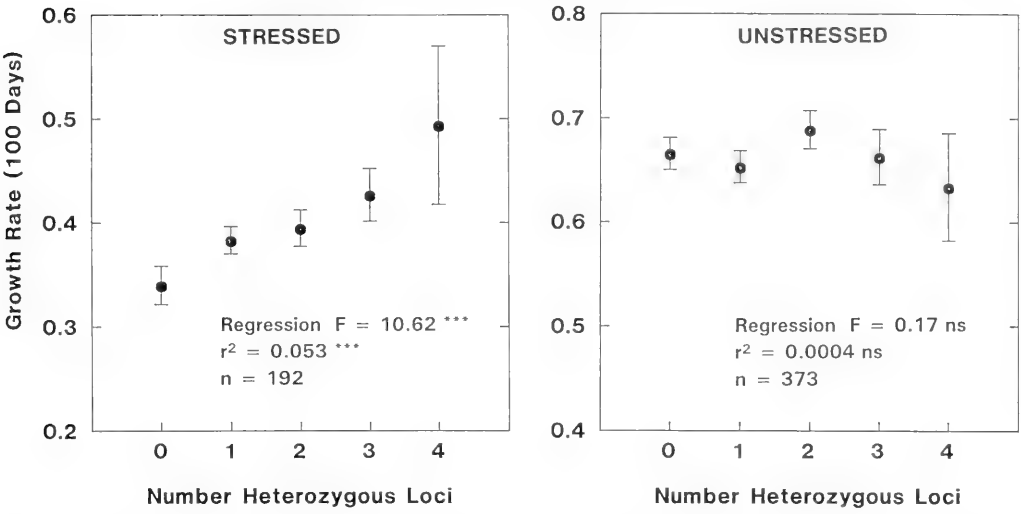

FIG. 1. Correlation and regression of individual juvenile growth rate and level of heterozygosity for the stressed and unstressed experimental cohorts. Data are depicted as means (± 1 SE) to illustrate the patterns, but the analysis is on individuals.

corresponding to Hardy-Weinberg expectations. The exception, ALA in Coweeta snails, exhibited a deficiency in heterozygotes ($D = -0.363$; $X^2 = 6.03$, $P = 0.014$). Two loci, MPI and ALA, were also monomorphic in Highlands but not Coweeta snails, contributing to the lower P-value in the Highlands' population. The overall F_{ST} (Nei 1977) for all loci (0.121) was significant ($X^2_{df=13} = 35.4$, $P < 0.001$). Mean F_{IS} and F_{IT} were positive and high (0.101 and 0.210 respectively).

Association Between Heterozygosity and Growth Rate

Effect of Stress at Level of Population (Cohort): The association between an individual's growth rate and its number of heterozygous loci was highly significant for the stressed cohort but not for the unstressed cohort (Fig. 1). Because of the large number of points in each treatment level, only the growth rate means (± 1 SE) for each heterozygote frequency value are shown in Figure 1. However, the values of r^2 and F are from the analysis of individuals, not means. Adding the data of the 6th locus (PGM-1) did not change the nonsignificant growth rate-heterozygosity association in the unstressed cohort. In addition, the growth rate of heterozygous individuals was 17% higher than fully homozygous individuals ($F_{1,190} = 6.622$, $P = 0.011$) in the stressed cohort. In the unstressed cohort, growth rates did not differ between the two genetic groups (homozygous and heterozygous rates were 0.666 ± 0.015 and 0.667 ± 0.011 respectively, $P = 0.94$).

Effect of Stress at Brood Level (Clutch-Sibs): Of the eight clutches in the stressed treatment, only one from a Highlands parent (H3) exhibited a significant association between individual growth rate and number of heterozygous loci (Table 2). Three broods in the stressed treatment, however, had no surviving homozygotes. The trend was towards greater growth rates of heterozygotes over homozygotes. In the unstressed cohort, only one brood lacked surviving homozygotes. One brood had a borderline significantly faster growth rate-heterozygosity association (Table 2), and again, there was a trend towards enhanced growth of heterozygotes over homozygotes in three of the remaining broods.

ANCOVA of individual growth rate among clutches (heterozygosity differences among clutches as covariate) indicated significant variation for both stressed and unstressed broods ($F_{7,183} = 26.62$, $P = < 0.0001$; $F_{7,364} = 14.61$, $P < 0.0001$ respectively). This suggests a strong parent or genotype effect on growth rate when differences in brood heterozygosity are controlled. Parent effects also significantly influenced the level of sur-

TABLE 2. Results of analysis of variance of growth rate by number of heterozygous loci for each *Mesodon normalis* brood in stressed and unstressed cohorts. The Differ. column represents the percentage difference in growth rate of heterozygous over fully homozygous individuals (+ value).

	Stressed					Unstressed			
Brood	Differ (%)	F	P	r^2	Brood	Differ. (%)	F	P	r^2
C1	no surviving homozygotes				C1	−2.6	0.43	0.52	0.009
C2	+8.3	0.04	0.85	0.001	C2	+27.5	3.78	0.06	0.078
C3	no surviving homozygotes				C3	−4.3	1.01	0.32	0.032
C4	+20.6	0.36	0.57	0.056	C4	+11.8	1.36	0.25	0.027
C6	−1.0	2.84	0.10	0.098	C5	no surviving homozygotes			
C7	no surviving homozygotes				C6	+39.9	4.07	0.05	0.085
H3	+20.0	5.93	0.02*	0.165	C7	+12.8	4.42	0.04*	0.121
H4	+12.0	0.587	.451	0.022	H1	−1.9	0.48	0.49	0.00

vival among broods in the stressed treatments ($X^2_{df=7}$ = 73.3, P < 0.0001) but not in the unstressed broods (P = 0.61).

The Influence of Site of Origin

Because genetic heterozygosity of adults differs between the two sites of origin, and level of survival in the stressed treatment was influenced by the variable parent, it is of interest to know if growth rate and heterozygosity of the young were also effected by site of origin. ANOVA confirms that both variables in both treatments are significantly higher in young from Coweeta parents than from Highlands parents (Fig. 2) (Growth: $F_{1,190}$ = 36.96, P = < 0.0001; $F_{1,371}$ = 8.27, P = 0.004; Hetloci: $F_{1,190}$ = 66.11, P < 0.0001; $F_{1,371}$ = 25.34, P < 0.0001).

The Contribution of Individual Loci

The relative contribution of each locus to the multiple-locus positive association of growth rate and heterozygosity in the stressed treatment was assessed by multiple regression (Table 3). The Type III sum of squares for a given locus is a measure of the association of heterozygosity with growth rate at that locus. The rankings of loci by their SS indicates the importance of two loci, ALA and PGI, with both being significant (Table 3). The highest ranking loci are not necessarily those with the highest level of heterozygosity. An analysis of differences in mean growth rates between homozygotes and heterozygotes at each locus (Fig. 3) confirmed the significant contribution of ALA and PGI to the positive fitness-heterozygosity association.

A comparable multiple regression analysis was also performed on the unstressed data set. Two loci, ALG and ALA, showed significant F-values. Further analysis indicated that in the ALA locus homozygotes exhibited a higher growth rate than heterozygotes, but for the ALG locus the converse was true.

Heterozygote Deficiencies and Offspring-Parent Genetic Relationships

Parents and their offspring from the same site displayed comparable levels of heterozygosity (Fig. 4). In the stressed cohort, only PGI exhibited a significant deficiency in heterozygotes (Table 4). However, in the unstressed cohort there were two loci (PGI and ALG) that had deficiencies, both in Coweeta snails and one (PGI) in Highlands snails. On pooling over sites, PGM-2 and ALA also became deficient. In adults from both sites, only one of the five loci showed a heterozygote deficiency (D-value for ALA was −0.417***, by chi-square) (Table 4). It appears, therefore, that in *Mesodon normalis* the finding of a significant association between growth rate and genetic heterozygosity is not associated with increased levels of heterozygote deficiency among loci. However, one of the two loci that showed a significant contribution to the correlation was also deficient in heterozygotes.

When the genetic structure of surviving offspring and their corresponding monoecious parent was compared, there was no evidence of selfing in any of the 16 adults; that is, non-parent alleles were present in the offspring of at least one locus in every adult. Evidence of multiple paternity was found in eight of the 16 adults by comparing the locus-specific genotype of the parent with all

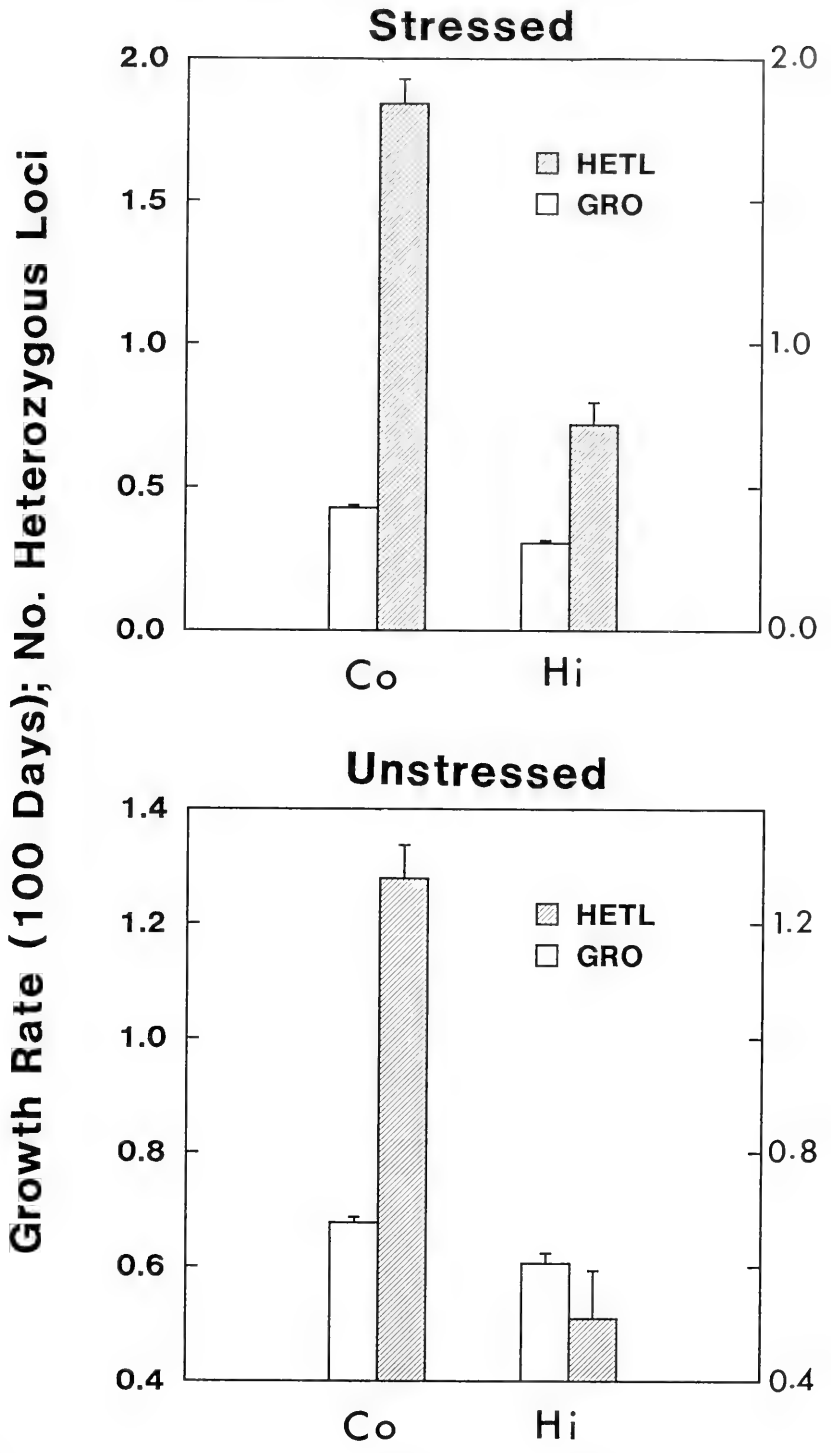

FIG. 2. Mean growth rates (left X-axis) and number of heterozygous loci (right X-axis) for Coweeta and Highlands clutches in the stressed and unstressed treatments. Error bars are 1 SE.

genotypes of all corresponding offspring. Multiple paternity may actually be higher because the genotypes of young that died during the experiment are not known.

Variation in Tissue and Shell Colors

The frequencies of the three tissue color classes did not differ between the Coweeta and Highlands young in either the stressed or unstressed cohort (Table 5). However, in the stressed cohort higher growth rates and higher levels of genetic heterozygosity were associated with the light tan morph, and significantly lower values with the intermediate and dark morphs. If these tissue color morphs have a simple genetic basis (Table 5), then the color morph frequencies conform to Hardy-Weinberg expectations in the stressed cohort but not in the unstressed cohort.

Shell color and tissue mottling were recorded only for individuals in the unstressed cohort. Brown shell morphs had their highest frequency (73%) in snails derived from Coweeta, and grayish brown morphs were the exclusive shell color in the Highlands snails. Genetic heterozygosity (number of heterozygous loci per individual) was significantly higher in brown shelled and mottled tissue morphs ($F_{1,371} = 43.57$, $P < 0.0001$; $F_{1,371} = 5.69$, $P = 0.018$, respectively). Growth rate did not differ between shell color morphs, but was higher in mottled than in non mottled morphs ($F_{1,371} = 46.60$, $P < 0.0001$).

DISCUSSION

The Growth Rate-Heterozygosity Association

The finding of a positive association between growth rate and allozyme heterozygosity in *M. normalis* at the population level parallels that of many similar studies (Allendorf & Leary, 1986; Zouros & Foltz, 1987). However, the positive association in *M. normalis* was found only in the stressed cohort, in which higher mortality and reduced mean growth rate occurred. In the control population in which survivorship and growth rate were significantly higher, growth rate was independent of genetic heterozygosity. In many of the studies depicting a positive association, information on possible stress conditions is often not available. As in many of these studies, the positive association in *Mesodon* occurred during the vulnerable early life stage of the cohort, a time when most energy is being allocated to somatic growth in mollusks (Zouros et al., 1980). However, for *M. normalis,* it is not known if the association eventually disappears as the snail cohort ages, as has been shown in some marine bivalves (Diehl & Koehn 1985). In *Mesodon,* the amount of variance in growth rate explained by variation in heterozygosity is small (5.3%), but corresponds to r^2 values from similar studies, even those in which the number of loci sampled was three times that of this study (Koehn et al., 1988).

TABLE 3. Results of multiple regression analysis on growth rate-heterozygosity association for each locus utilizing all individuals in the stressed cohort. H is the mean direct-count heterozygosity value for all individuals for each. Loci are ranked in their importance by value of the Type III sum of squares (SS). $R^2 = 0.103$ and overall regression $F_{4,186} = 4.28$, $P = 0.001$.

Locus	H	SS	F	P
APA	0.54	0.148	10.54	0.001***
PGI	0.34	0.068	4.86	0.029*
PGM-2	0.06	0.010	0.71	0.400
MPI	0.02	0.002	0.14	0.710
APG	0.55	0.001	0.07	0.793

*P < 0.05, ***P < 0.001

The *Mesodon* results are also consistent with those of the few studies in which environmental stress was described or was part of a planned experimental treatment. In some studies, increased cohort mortality was associated with stress (Samollow & Soule, 1983). Such was the case in this study. Also individuals that have a greater probability of dying younger are those that are slower growers and hence smaller (Foster & Stiven, in review), thus leaving a greater proportion of larger, more heterozygous and faster growing individuals. These results for *M. normalis* appear to be consistent with the overdominance model, where multiple locus heterozygotes exhibit superior fitness to their associated homozygotes (Zouros & Foltz, 1987; Zouros et al., 1988).

For this laboratory scenario to be relevant to natural *Mesodon* populations, a group of hatchlings or juveniles would have to be exposed to a period(s) of environmental stress or crowding that causes mortality (e.g. a cold, wet period with reduced dispersal ac-

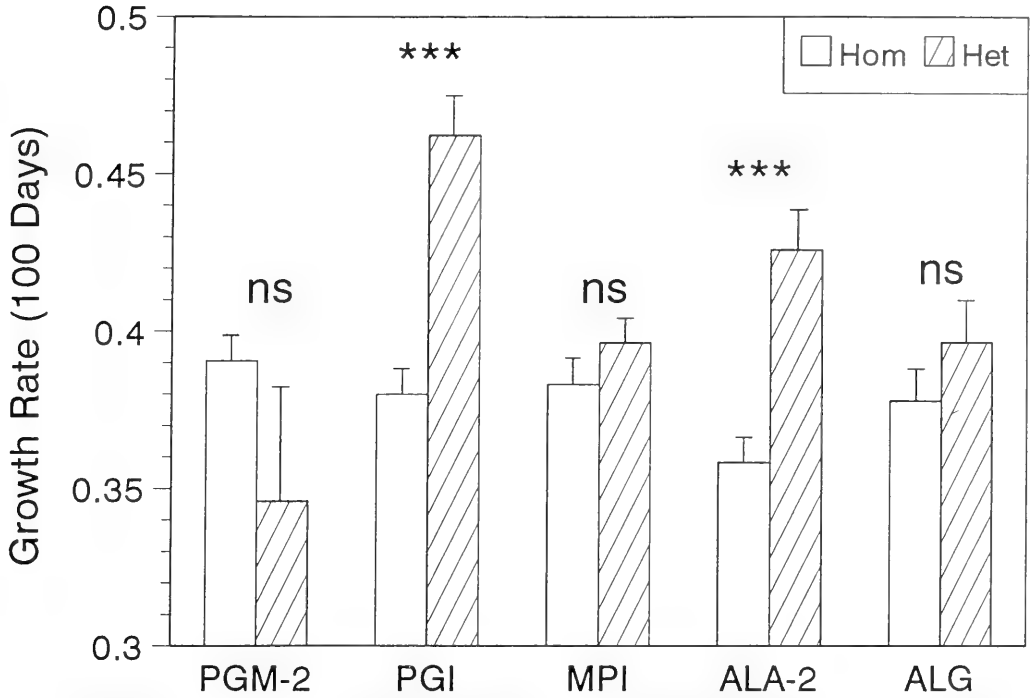

FIG. 3. Mean growth rates for homozygous and heterozygous individuals for each locus in the stressed cohort. Error bars are 1 SE, ns means not significant at P = 0.05, *** means significant at P < 0.001 (t-tests).

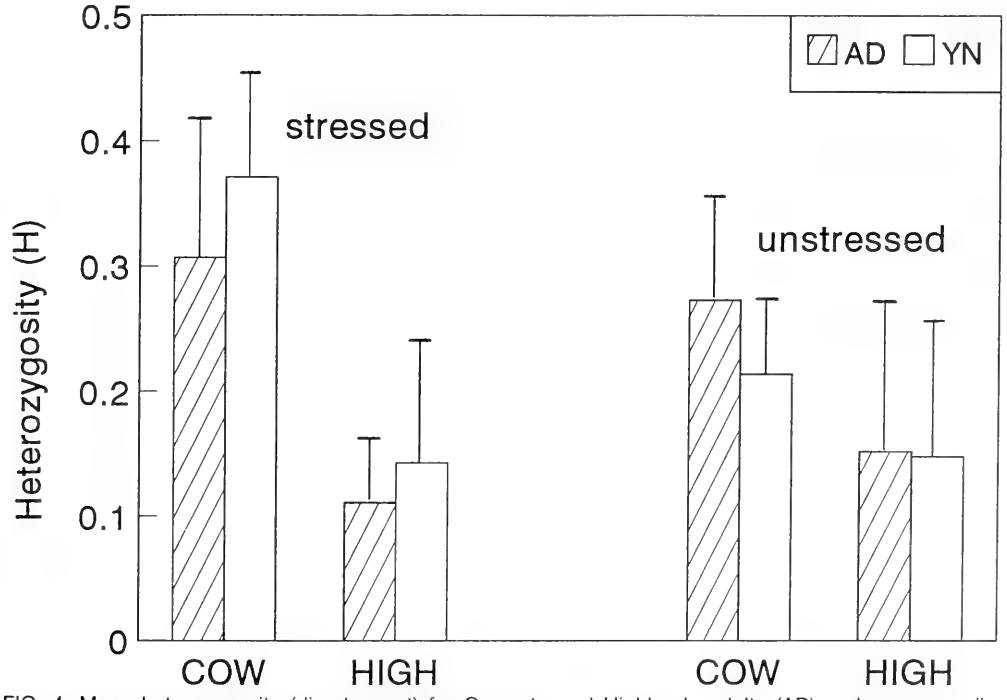

FIG. 4. Mean heterozygosity (direct count) for Coweeta and Highlands adults (AD) and corresponding offspring (YN) for the stressed and unstressed treatments. Error bars are 1 SE.

TABLE 4. Heterozygote deficiencies (D) for loci in the stressed and unstressed cohorts of surviving young. Significance by chi-square. COW and HIG are the Coweeta and Highlands sites.

Cohort		PGM-2	PGI	MPI	ALA	ALG
Stressed:	COW	0.028	−0.710***	0.260	0.169	−0.110
	HIG	0.017	−0.740**	0.037	−0.098*	0.293
	POOLED	0.022	−0.723***	0.193	0.033	−0.105
Unstressed:	COW	0.009	−0.332***	−0.022	−0.074	−0.205***
	HIG	−0.153	−1.000***	0	0	0
	POOLED	−0.393***	−0.417***	−0.037	−0.098*	−0.398***

*P < 0.05, ** 0.05 < P < 0.01, ***P < 0.001

TABLE 5. Comparisons of tissue and shell color properties of surviving young in the stressed and unstressed cohorts.

Comparison	Unstressed	Stressed
Tissue Color*		
Between sites	ns	ns
Growth among colors	ns	$F_{2,189} = 5.21$, $P = 0.006$ $1 < 2, 2 = 3$
Heterozygosity among colors	ns	$F_{2,189} = 4.21$, $P = 0.016$ $1 < 3, 2 = 3$
Hardy-Weinberg conformity**	$X^2 = 67.5$, $P < 0.001$	ns
Shell Color*		
Between sites	$X^2 = 57.62$, $P < 0.0001$	not recorded
Growth between colors	ns	not recorded
Heterozygosity between colors	$F_{1,371} = 42.61$, $P < 0.0001$ $1 < 2$	not recorded
Tissue Mottling*		
Between sites	$X^2 = 8.69$, $P = 0.003$	not recorded
Growth between classes	$F_{1,371} = 46.6$, $P < 0.0001$ $2 > 1$	not recorded
Heterozygosity between classes	$F_{1,371} = 6.69$, $P = 0.018$ $1 > 2$	not recorded

*Tissue color: 1 = light tan, 2 = intermediate, 3 = dark
*Shell color: 1 = brown, 2 = greyish brown
*Tissue mottling: 1 = none, 2 = mottled
**Assuming A_1A_1 = color 1, A_1A_2 = color 2, A_2A_2 = color 3

tivity, mucus accumulation and high litter moisture, or a late frost). Unfortunately, little is known of mortality and its causes in *Mesodon normalis* in the field. Mortality in the laboratory is density dependent, and in the field mean adult size is larger in low density environments (Foster & Stiven, in review), as is the case in *Capaea nemoralis* (Oosterhoff, 1977).

When the growth rate-heterozygosity association was examined separately in each of the eight clutches in the stressed treatment, only one was positive. However, in three of the remaining clutches, only heterozygotes were found at the end of the experiment, suggesting that the homozygotes died, although the initial frequencies of genotypes could not be assessed. The non-significant trend in the remaining clutches was for higher growth rates in heterozygotes. A smaller sample size (brood vs. cohort) may also be partly responsible for the lack of significance (Beaumont et al., 1983; Zouros & Foltz, 1987), or there may be a true absence of the association at the brood level. In a number of other studies of sibling cohorts in marine bivalves (Beaumont et al., 1983; Gaffney & Scott, 1984; Beaumont et al., 1985; Mallet et al., 1986), positive relationships were also absent. Gaffney & Scott (1984) point out that many of the positive associations between growth and heterozygosity in marine bivalves come from large populations, from which individuals were sampled at random, and that individuals coming from a single mating may not show the positive association. The prob-

lem of detecting the positive relationship at the brood level might also be affected by the number of different matings of the parent with different adults, as well as differences in survivorship among clutches (Zouros & Foltz, 1987). In *Mesodon,* the removal of possible effects of differential clutch survivals levels by ANCOVA did not abrogate the positive association at the population level in the stressed cohort, nor did it change the outcome when the level of analysis was by brood rather than across individuals.

In the stressed *Mesodon* cohort, two of the five loci (ALA, PGI) were significant contributors, with heterozygotes having faster growth rates than homozygotes for both loci. PGI functions in the glycolytic pathway, and ALA is involved in protein catabolism. In the Koehn et al. (1988) study of the coot clam, *Mulinia* loci, ALA was significant but PGI was not; a total of eight out of 15 loci had significant effects, including PGM, MPI and three nonspecific AP loci. Gentili & Beaumont (1988) reported significant contributions of only two out of eight loci in a high density treatment cohort of *Mytilus edulis,* and Borsa et al. (1992) found that only one locus (PGM) out of seven had a higher heterozygosity in the survivors of a marine bivalve exposed to anoxic stress compared to the control. In the unstressed *Mesodon* cohort, two loci had contrasting relationships; growth rate was higher in heterozygotes in one locus but lower in heterozygotes in the other. Most studies of the assessment of the comparative contribution of loci have utilized up to five or six loci. Koehn et al. (1988) warned that this number may be inadequate. They argued that a large enough sample of diverse polymorphic genes should be assayed to encompass various metabolic roles, that the linkage relationship among loci be known, and that the correlation between a fitness parameter and heterozygosity be established. Whereas the last assumption is met in the *Mesodon* study, and the polymorphic loci used cover a range of functions, the number (5) is small, and linkages among the loci are not known. Thus, it is premature to draw definitive conclusions about which loci are more significant contributors to the heterozygosity-growth rate relationship in the *Mesodon* system.

The significant F_{ST} value of 0.121 for the adult sample (those producing clutches plus those that did not) from Coweeta and Highlands sites suggests sizeable differentiation (Wright, 1978; Hartl & Clark, 1989). The high positive values of F_{IS} and F_{IT} also reflect the high levels of homozygosity in the populations, expected in a species with limited dispersal and probably inbreeding. These F_{ST} values, although derived from relatively low sample numbers are similar to those reported for two other land snails, *Mesomphix andrewsae* and *Mesomphix subplanus*, from separate watershed in the Coweeta forest (Stiven, 1989).

Zouros & Foltz 1987 suggest that the presence of many loci with heterozygous deficiencies is associated or even enhances the chance of finding a positive correlation of growth rate and heterozygosity. In the stressed *Mesodon* cohort, which exhibited the positive growth rate-heterozygosity association, only one locus (PGI) showed genotypic frequencies that did not conform to Hardy-Weinberg expectations, and this locus was a significant contributor to the association (Table 3). In contrast, two loci showed heterozygote deficiencies in the unstressed control treatment. Therefore, in this study the presence of loci that are deficient in heterozygotes was not a necessary condition for a significant fitness-heterozygosity association.

How balanced are the stressed and unstressed experimental treatments from the two different years with regard to site of origin and genetics of contributing parents? The ratio of Coweeta to Highlands parents was very similar, 8:1 and 8:2 for the stressed and unstressed treatments, respectively. As noted earlier, the heterozygosity level in Coweeta adults was over twice that in Highlands adults. However, mean heterozygosities for the stressed and control treatments were similar, 0.233 and 0.250. It appears, therefore, that initial parental genetic diversity and site contribution are essentially equivalent in the two treatments. However, more population genetic and ecological work should focus on understanding the causes of the different levels of heterozygosities as well as the different mean clutch sizes found between Coweeta and Highlands.

Significance to Population Processes in *Mesodon normalis*

Part of the population regulation process in the terrestrial gastropod *M. normalis* comes from the effects of population density on juvenile growth and their subsequent rate of

development to adults. In the field, adult densities and adult sizes are negatively correlated, with Coweeta sites having higher densities and smaller adult sizes than Highlands sites (Foster & Stiven, in review). As in many invertebrates, growth rates are quite variable among juveniles, and this variability has significance for both final size and time and age at maturity. From our laboratory experiments on the effects of density and food (Foster & Stiven, in review), a small fraction (5.1%, or 11 animals) ceased growth and showed adult characteristics after one year of growth. These were the faster growers. Also those growing longest tended to be those that were smallest at the beginning of the experiment. In the field, juveniles that were larger at the start of the second winter also became the larger adult the following summer, the usual time of first breeding (Foster & Stiven, in review), and these would be the faster growing juveniles. They may produce their first offspring that summer, and may live up to three years more as adults (Foster & Stiven, in review). The smaller and slower growing juveniles are also more prone to die younger under increased environmental stress (i.e. density; Foster & Stiven, in review) and are also the more homozygous individuals (this study). "Older" adults, regardless of size, also produce twice as many clutches but fewer eggs per clutch as do the "younger" adults, but the total number of eggs and hatching success do not differ with age (or adult body size) (Foster & Stiven, in press). Adult age cannot be precisely determined, but the older adults have extensively eroded periostraca, whereas younger adults have intact periostraca. If, under stress conditions, the faster growing more heterozygous juveniles have better survivorship, mature and reproduce sooner (possibly even at the end of the second summer, but at least early the next summer), their lifetime fitness would obviously be greater, and they may become larger adults. It is not known if the smaller adults derived from the slower growing, more homozygous juveniles would also be more prone to higher mortality after reaching adulthood. In the European helicid snail *Cepaea nemoralis,* the larger faster growing juveniles tend to reach sexual maturity earlier and to be the larger adults (Oosterhoff, 1977). In addition, parental body size, egg size and fecundity, and juvenile growth rate are positively correlated (Oosterhoff, 1977; Carter & Ashdown, 1984; Baur, 1988). In contrast, in *M. normalis,* juvenile growth rate and reproductive output are independent of the size of the parent, even though clutch number and clutch size are related to adult age (Foster & Stiven, in press).

Therefore, the significance of the variable and genotype-dependent growth and mortality rates in *M. normalis* may lie not so much with differential fecundity, but with a coupling of increased survival and earlier reproduction for the more heterozygous faster growers, especially when adverse environmental conditions occur during the period of juvenile growth.

Breeding System

No evidence of selfing was apparent from a comparison of parent and offspring genotypes in *M. normalis* from either site, confirming the speculation of Foltz et al. (1984) that native gastropods in relatively undisturbed environments would be outcrossers, with self-fertilization the more likely mode of the colonizer (European).

There is also strong evidence (62.5% of the parents) for multiple paternity in *M. normalis*. While perhaps widespread in pulmonate mollusks, most reports, such as this, come from studies with another focus (Murray, 1964).

Tissue and Shell Color Morphs

Whereas the frequencies of the three tissue color morphs did not differ between the stressed and unstressed treatments, higher growth and heterozygosity levels were characteristic of the light tan morph, with significantly lower values for the dark and intermediate forms. The significance of this is not known, but the morph frequencies in both Coweeta and Highlands offspring from the stressed cohort conform to Hardy-Weinberg expectations, suggesting a genetic mechanism. Tissue color, tissue mottling, and shell color variation in these populations require further work, especially as markers in population work.

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DIAGNOSIS OF THE GENUS *CRASSOSTREA* (BIVALVIA, OSTREIDAE)

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ABSTRACT

The oyster genus *Crassostrea* is the only valid genus in the subfamily Crassostreinae. Characters or tendencies considered diagnostic of other crassostreine genera are either environmentally controlled or can be found in the type species, *C. virginica,* or in its direct forerunner, *C. gigantissima.* Late larval forms of this genus all possess distinctive convexities and ligament placement; adults have a right side promyal passage and a nonorbicular adductor muscle scar. A large size and elongate outline, a cupped left valve, an umbonal cavity, posterior and/or ventral displacement of the muscle scar, large void chambers, and significant nonvesicular chalky deposits are skeletal characters that may be present in some populations or species of this extremely variable taxon. *Crassostrea* as rediagnosed has few living species; the taxon is evolutionarily conservative. Applying this conservatism to the fossil record, recognizing that chomata are a part of the history of the genus, and realizing that similar evolutionary changes have not been synchronous throughout the geographic range of the genus, are all essential to deciphering the geologic history and evolution of *Crassostrea.* Ongoing and future biological work should contribute significantly to the understanding of this history.

Key words: Bivalvia, Ostreidae, Crassostreinae, *Crassostrea,* taxonomy, classification.

"The first prerequisite in oyster classification is availability of ample material." (Stenzel, 1971: N1094)

INTRODUCTION

Stenzel (1971) suggested a diphyletic origin for the oysters and recognized two families, the Gryphaeidae Vyalov, 1936, and the Ostreidae Wilkes, 1810, within the superfamily Ostreoidea Wilkes, 1810 (Table 1A). Polyphyly might be inferred from the subsequent creation of some additional ostreoidean families [e.g. for *Crassostrea* and its allies by Scarlato & Starobogatov (1979a, 1979b) and for the pycnodonteine oysters by Torigoe (1981)]. However, these and other proposals have not been accompanied by clear indications of the evolutionary significance of the taxa involved. By contrast, Malchus (1990), in a work emphasizing Cretaceous oysters, erected a third ostreoidean family, Palaeolophidae (Table 1B), and used interpretations of shell structures to suggest the historical significance of his taxon. Although arguments for monophyly have been made (Nicol, 1984), and the most recent synopsis of living oysters (Harry, 1985) maintains a two-fold division of the group, Malchus's still more recent work proffers triphyletic origins for the oysters.

Harry (1985) recognized three subfamilies within the Ostreidae: Lophinae Vyalov, 1936; Ostreinae Wilkes, 1810; Crassostreinae Scarlato & Starobogatov, 1979 (Table 1A). Harry further pointed out that the separation of *Crassostrea* and its associates at the subfamily level had been presaged by the arrangement of taxa in Stenzel (1971). Malchus (1990) erected a fourth subfamily for Jurassic-Cretaceous ostreid oysters: subfamily Liostreinae Malchus, 1990 (Table 1B). The present study questions the groupings of genera within this latter subfamily, and also queries Malchus's placement of genera within the Crassostreinae.

One traditional evolutionary view has the crassostreine oysters derived from the ostreine members of the family during the Cretaceous Period as oysters supposedly moved into more inshore and coastal settings (Yonge, 1960: 97). Yet the Crassostreinae possess characters that many workers deem to be primitive in nature (discussion in Stenzel, 1971: N1958), suggesting that the geologic history of these two subfamilies may be the reverse of that proposed by Yonge. Malchus (1990) has begun the application of primitive-versus-derived character recognition (Henning, 1966; Ax, 1987; Funk & Brooks, 1990) to the oysters. A clear under-

TABLE 1. Families and subfamilies within the superfamily Ostreoidea Wilkes, 1810, with their general geologic ranges. Comparison of pre-1990 classification (A) with that proposed by Malchus in 1990 (B). Subfamilies with living representatives marked by an asterisk (*).

A. Pre-1990 oyster classification. After Stenzel (1971), Torigoe (1981), Freneix (1982), and Harry (1985).

Family Gryphaeidae Vyalov, 1936; Triassic-Neogene
  Subfamily Gryphaeinae Vyalov, 1936; Triassic-Jurassic
  Subfamily Exogyrinae Vyalov, 1936; Jurassic-Cretaceous
  Subfamily Gryphaeostreinae Freneix, 1982; Cretaceous-Neogene
  *Subfamily Pycnodonteinae Stenzel, 1959; Cretaceous-Neogene
Family Ostreidae Wilkes, 1810; Triassic-Neogene
  *Subfamily Lophinae Vyalov, 1936; Triassic-Neogene
  *Subfamily Crassostreinae Scarlato & Starobogatov, 1979; Cretaceous-Neogene
  *Subfamily Ostreinae Wilkes, 1810; Cretaceous-Neogene

B. Classification of Malchus (1990: p. 196, adapted from table 17).

Family Palaeolophidae Malchus, 1990; Triassic-Cretaceous
  Subfamily Palaeolophinae Malchus, 1990; Triassic-Cretaceous
Family Gryphaeidae Vyalov, 1936; Triassic-Neogene
  Subfamily Gryphaeinae Vyalov, 1936; Triassic-Jurassic
  Subfamily Exogyrinae Vyalov, 1936; Jurassic-Cretaceous
  Subfamily Gryphaeostreinae Freneix, 1982; Cretaceous-Neogene
  *Subfamily Pycnodonteinae Stenzel, 1959; Cretaceous-Neogene
Family Ostreidae Wilkes, 1810; Triassic-Neogene
  Subfamily Liostreinae Malchus, 1990; Triassic-Neogene
  *Subfamily Lophinae Vyalov, 1936; Paleogene-Neogene
  *Subfamily Crassostreinae Scarlato & Starobogatov, 1979; Paleogene-Neogene
  *Subfamily Ostreinae Wilkes, 1810; (?)Paleogene-Neogene

standing of the taxa involved, and most especially of within-taxon variability, is critical to this technique. Hopefully, this paper will encourage the use of phylogenetic viewpoints through an analysis and diagnosis of the genus *Crassostrea*.

BACKGROUND: CHARACTERS OF THE CRASSOSTREINAE

Stenzel (1971), in the *Treatise on Invertebrate Paleontology,* provided an historical summary of many of the most important works in ostreid taxonomy. Stenzel, with a background of work including studies by Orton (1928), Nelson (1938), Ranson (1943, 1948), Stenzel (1947), Gunter (1950), Thomson (1954), and Sohl & Kauffman (1964), contended that both the anatomy of living oysters and the shell characters of living and fossil oysters must be considered in the development of any meaningful classification. When dealing with exoskeletons alone, Stenzel argued for primary reliance upon shell microarchitecture and for the relative taxonomic importance of features on the surface of the shells' internal cavity, because changes in the latter characters may often reflect alterations in the position of soft tissues.

By the time Stenzel wrote the *Treatise* volume, workers had recognized numerous ostreid characters with real or potential value in unraveling the systematic and evolutionary relationships of the group. These features included larval development and form; adult shell outlines and relative sizes; valve geometries; the presence or absence of a promyal passage on the right side of the soft tissues; the geometry and placement of the adductor muscle; and the degree of development of left valve umbonal cavities and also of valve chambers, both void and containing chalky deposits.

Among these characters, nonincubatory larval development; a late larval or prodissoconch II shell with a distinctly more convex left valve and a ligament developed far anterior to any tooth precursors; the presence of a right side promyal passage; and a nonorbicular adductor muscle scar are presently recognized as invariant attributes of crassostreine oysters (Stenzel, 1971; Torigoe, 1981; Freneix, 1982; Harry, 1985; see later section for discussion of larval form; contrast Malchus, 1990: 82, table 9, for muscle scar). Other soft-tissue and unifying characters of the Crassostreinae include thickened and food-storing mantle lobes and an accessory heart that "does not receive

TABLE 2. Nominal genera within subfamily Crassostreinae Scarlato & Starobogatov, 1979, and characters that have been suggested as distinguishing the other genera from *Crassostrea* Sacco, 1897. Discussion of these character differentiations in text. Data from Stenzel (1971), Chiplonkar & Badve (1979), Torigoe (1981), Harry (1985), Chinzei (1986), and Moore (1987).

CRASSOSTREINE GENERA

1—*Crassostrea* Sacco, 1897; Cretaceous-Holocene
2—*Pseudoperna* Logan, 1899; Cretaceous
3—*Acutostrea* Vyalov, 1936; Cretaceous-Eocene
4—*Gyrostrea* Mirkamalov, 1963; Cretaceous
5—*Indostrea* Chiplonkar & Badve, 1976; Cretaceous
6—*Bosostrea* Chiplonkar & Badve, 1978; Cretaceous
7—*Soleniscostrea* Chiplonkar & Badve, 1979; Cretaceous
8—*Cussetostrea* Chiplonkar & Badve, 1979; Cretaceous
9—*Konbostrea* Chinzei, 1986; Cretaceous
10—*Striostrea* Vyalov, 1936; Eocene, Holocene
11—*Saccostrea* Dollfus & Dautzenberg, 1920; Miocene-Holocene

COMPARISON OF OTHER GENERA WITH *CRASSOSTREA*

Character in Comparison with *Crassostrea*		Genus, by numbers above									
		2	3	4	5	6	7	8	9	10	11
A.	Denticles or chomata present	X	X	X	X		X	X		X	X
B.	Adult shell size smaller/larger	X	X		X				X		
C.	Greater valve massiveness									X	
D.	Umbonal/ligamental areas different	X	X	X	X					X	X
E.	Conical shell form present								X	X	X
F.	External ornamentation different				X	X		X		X	
G.	Muscle scar placement different							X		X	
H.	Muscle scar geometry different									X	X

adjacent neobranch units as tributaries" (Harry, 1985: 149).

The Crassostreinae may also display a relatively large size and generally elongate outline among oysters, a cupped left valve and flatter right valve, an umbonal cavity under the ligamental area of the left valve, and a posterior and/or ventral displacement of the adductor muscle and its scar; they have the ability to produce internal and large valve chambers, which may be filled with nonvesicular chalky deposits (Gunter, 1950; Torigoe, 1981; Harry, 1985; Malchus, 1990). [Chalky deposits of crassostreine oysters are nonvesicular at any magnification of light microscopy, but apparently display "microvesicles" under the electron microscope; Carriker et al., 1980; Harry & Dockery, 1983).] Yet none of these latter characters is constant in expression within the taxa of this subfamily. Extreme within-taxon variability of crassostreine oysters had been recognized for many years prior to the appearance of the *Treatise* volume (for example, Korringa, 1952; Gunter, 1954), and parts of this variability were acknowledged by Malchus (1990) in the most recent summary classification of the oysters.

Unfortunately, Malchus (1990: 99, 101) did not formally define his concept of the Crassostreinae, but various text discussions and tables are present (esp. pp. 68–98, 196) from which his notions can be extracted. Malchus used shell microstructure as his primary taxobasis. Within this framework, the Crassostreinae were characterized by having primarily simply foliated layers in the inner ostracum (non-prismatic layer) of their shells; these layers are arranged in an extremely lenticular fashion and form half or less of a typical valve cross-section. The remaining spaces are chambers, typically large, which may be void or be filled with chalky deposits. The Crassostreinae share these basic characters with the Ostreinae, even though minor structural differences within and between the subfamilies are present (Malchus, 1990: 69, 87).

This microstructural basis caused Malchus to reassign genera within the Ostreidae. Prior to 1990, at least eleven genera had been proposed that could be assigned to the Crassostreinae (Table 2, top, 1–11). The majority of these taxa had been erected for Cretaceous representatives. Malchus (1990: 196; Table 1B) apparently removed all of these

Cretaceous genera from the crassostreinae and referred many of them to his new subfamily Liostreinae; this latter subfamily he characterized as having no or few, mostly small chambers that may be void or chalk-filled. Malchus (1990: 201) did not treat the Cretaceous *Crassostrea*-like genus *Konbostrea* Chinzei, 1986, or the similar-aged genera *Soleniscostrea* Chiplonkar & Badve, 1979, and *Cussetostrea* Chiplonkar & Badve, 1979. *Konbostrea* is characterized by pre-eminent, large, lenticular areas filled with altered chalky deposits and cannot be assigned to Malchus's Liostreinae.

The *Crassostrea*-like genera that Malchus removed to the Liostreinae [*Pseudoperna* Logan, 1899; *Acutostrea* Vyalov, 1936; *Gyrostrea* Mirkamalov, 1963; *Indostrea* Chiplonkar & Badve, 1976; *Bosostrea* Chiplonkar & Badve, 1978] all include taxa with relatively small adult stages. If lineages of oysters are marked by increases in adult sizes (see following section) then Malchus's (1990: 75–76) own arguments, involving efficiency of chamber use in the building of larger or more elongate oyster exoskeletons, can be used to suggest that chambering (chalk-filled or void) should also increase in prominence and size through time. At least in the *Crassostrea*-like oysters, to arbitrarily subdivide them, using the degree and size of chambering as one primary taxobasis, is to create a "horizontal" classification (Newell, 1965) that does not aid the reconstruction of phylogenetic histories. Concomitantly, accepting the placement of *Turkostrea* Vyalov, 1936, and its allies, in the Crassostreinae (Malchus, 1990: 196) must await more formal reanalyses of these Cenzoic and largely Eastern Hemisphere taxa. These latter taxa have form characters different from those in previously recognized members of the subfamily Crassostreinae. Thus, the rest of this paper's discussion does focus upon the 11 genera of Table 2.

CRASSOSTREINAE AND *CRASSOSTREA*

Introduction

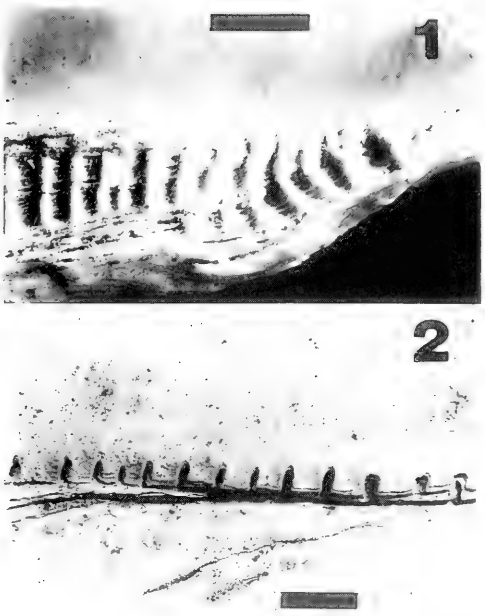
What distinguishes *Crassostrea* from the other ten genera on Table 2? Or stated conversely, what criteria or characters have been proposed to separate these other genera from *Crassostrea*? Distinguishing traits, as suggested by original definers or subsequent and major revisers, are outlined in Table 2 (bottom, A-H). These latter characters form the basis for comparisons with *Crassostrea* in the following portions of this section.

The supposedly distinctive features of the other ten genera include characters mentioned previously as variable within the subfamily, and involve shell size, massiveness, outline and form; external sculpture of both valves; the presence of denticles or chomata; and such features of the internal shell cavity as muscle placement and muscle scar outline. Are these valid distinguishing characters? To answer this question, the type species of *Crassostrea* must be examined, for "the type species must be elucidated fully first, else the genus [will] remain obscure" (Stenzel, 1971: N1095).

Crassostrea virginica and its History

Gunter (1950) and Stenzel (1947, 1971) have summarized the nomenclatural history of the species now known as *Crassostrea virginica* (Gmelin, 1791). By original definition, this extant species is the type species of *Crassostrea* Sacco, 1897. *Crassostrea virginica* is rather widespread in the western Atlantic Ocean, occurring from the coasts of the Maritime Provinces of Canada south through the Gulf of Mexico and Caribbean Sea to the coast of Brazil (Harry, 1985). Because of its economic importance, this species has been intensively and extensively studied for well over 100 years, with one summary of much of this work on the American (or Atlantic) oyster provided by Galtsoff (1964). Whenever practicable in the following discussions, terms and examples or illustrations are drawn from Galtsoff (1964) and Stenzel (1971) for living oysters, and from Stenzel (1971) and Malchus (1990) for fossil representatives; hopefully, the arguments may then be followed with the minimum of outside sources.

In examining the proposed distinguishing characters of crassostreine genera, the phylogeny of the type species is important, for living taxa are indeed the products of history. Only two proposals for the ancestry of *Crassostrea virginica* have been proffered. Sohl & Kauffman (1964) argued that the American oyster is the extant member of a lineage—their *C. soleniscus* (Meek, 1871) lineage—that began during the Cretaceous Period, and that the large and massive Tertiary oyster *C. gigantissima* (Finch, 1824) was the direct precursor of the extant *C. virginica.* Hopkins (1978), in a published abstract, suggested



FIGS. 1–2. Chomata on right valves of *Crassostrea gigantissima* (Finch) from (1) the late Eocene of Burke County, Georgia, and (2) the late Oligocene-early Miocene of Onslow County, North Carolina. Localities in text. Views of dorsoposterior right valve margins; valve interior up and dorsal axis to right. 1. Specimen N1-301, showing relict chomata on margin of valve adjacent to, but outside of, plane of commissure, bar = 2.0 mm. 2. Specimen LBC-28, view onto commissural shelf, showing raised rims and slit-like appearance of chomata, bar = 2.0 mm.

that the ancestry of *C. virginica* included the Late Cretaceous species *C. glabra* (Meek & Hayden, 1857). Among other reasons, Hopkins chose *C. glabra* because of its widespread occurrence in settings interpreted as brackish water, and he suggested that this ancestry may explain the extreme tolerance of *C. virginica,* among oysters, to waters of lowered salinities. Unfortunately, there is no written record of Hopkins' views of the immediate precursor of *C. virginica.* But, to assume static environmental tolerances through time is tenuous (in an evolutionary sense) and is counter to well-documented cases of Cenozoic oysters assigned to *Crassostrea* that lived in rather normal marine settings (e.g. Jimenez et al., 1991).

These arguments aside, *Crassostrea gigantissima* is the only crassostreine oyster available to serve as a direct forerunner for the American oyster. This giant, fossil oyster is widespread in the Western Hemisphere and its synonymy when completed (Lawrence, in preparation) should approach at length that of its related Eurasian species *C. gryphoides* (Schlotheim, 1813; see references in Stenzel, 1971: N1082). That transatlantic migrations provided the first stocks of *C. virginica* is highly unlikely for two reasons. First, the distances involved are too great for direct migration. The maximum cited larval dispersal distance in oysters is 1,300 km (Stenzel, 1971: N1035); the nearest coastal regions of the Eastern Hemisphere in Europe or Africa to the critical middle-Atlantic coast of North America during early Miocene times (see following section on chomata) are farther distant according to recent Atlantic Ocean basin reconstructions (Allmon, 1990: 111). Secondly, it is clearly nonsimplistic to call upon some special case of dispersal to happen at an exact and prescribed time in the past, especially when there is no evidence to support these migrations. Endemic Western Hemisphere crassostreine oysters are much more likely predecessors of *C. virginica.* Thus, the suggested differences between and among crassostreine oyster genera are examined below using both *C. virginica* and *C. gigantissima.* Because of their previous emphasis in the definitions of genera, denticles or chomata (Table 2, bottom, A) are addressed first.

Purported Diagnostic Characters of Other Crassostreine Genera

Chomata: Chomata are ridgelets or tubercules (right valves; Figs. 1, 2) and pits (left valves) that occur on or near the borders of inner valve surfaces (Stenzel, 1971: N1029, figs. J7, J30, J31, J84, J113, J127, J128, J129). Their origin and significance are still not understood. Within the Ostreidae, Malchus (1990: 87) recognized thin and unbranched steg-chomata and the reduced pustular chomata, with these features appearing on or near the free growing valve margin or lateral to the ligamental area (in the latter case, as relict chomata). Malchus's useful phrase "relict chomata" may be formally redefined to include chomata that do not appear on the latest formed lamellar layers, occupy very marginal positions, and are typically (but not invariably) lateral to the ligamental area.

Stenzel denied the presence of chomata in *Crassostrea;* this diagnosis has been ac-

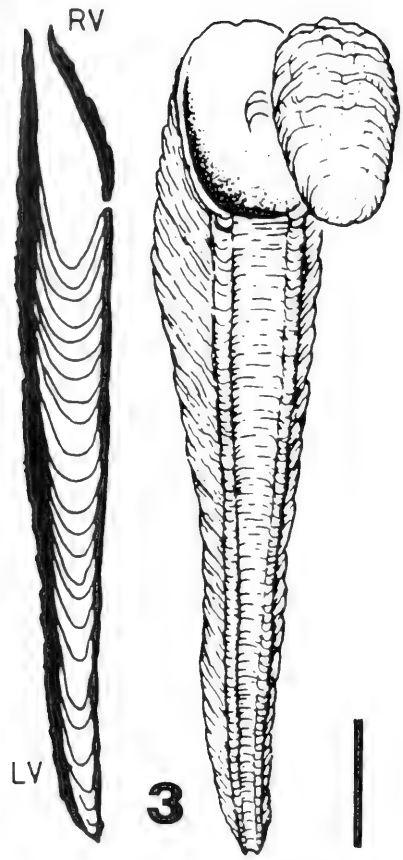


FIG. 3. *Saccostrea* sp., showing smaller, lid-like right valve and elongate left valve with prominent umbonal cavity and numerous void chambers internal to the ligamental area. Adapted from part of Chinzei (1982: fig. 15), bar = 5.0 cm.

cepted by Torigoe (1981), Harry (1985), and Malchus (1990) and has been used to distinguish *Crassostrea* from numerous other crassostreine genera (Table 2, bottom, A). But not all workers have shared this perspective upon the genus *Crassostrea.* Among neontologists, Thomson (1954: especially pp. 162–163) included chomata-bearing taxa in *Crassostrea.* Stenzel (1971: N1094) dismissed this view by calling the work of Thomson a "lumping" classification.

More pointedly, other paleontologists with access to large collections of Western Hemisphere Cretaceous and Cenozoic oysters have not considered the absence of chomata to be a distinguishing character of *Crassostrea* (e.g. Sohl & Kauffman, 1964; Woodring, 1982). Sohl & Kauffman's view of the *C. soleniscus* lineage has the taxa increasing in size and massiveness through mid-Cenozoic times, with the more recent *C. virginica* being smaller and more variable, and a decrease or gradual loss of chomata in this lineage through time. Stenzel (1971: N1028, N1193) was most certainly aware of Sohl & Kauffman's work but remained curiously silent on this differing concept of the genus *Crassostrea,* maintaining his position that "the underlying idea that chomata are a feature important to classification is sound" (Stenzel, 1971: N1088).

More recent work has supported this contrary view of the genus: "It would be unrealistic to suppose that the chomata-bearing valves represent a different species, much less a different genus. Wherever they were found, they are associated with valves that lack chomata. Three chomata-bearing valves are attached to the exterior of a right valve that lacks chomata. . . . Aside from the chomata, the two sets of valves are indistinguishable" (Woodring, 1982: 611–612; discussion of the Panamic and Cenozoic species *Ostrea cahobasensis* Pilsbry & Brown 1917, which Woodring assigned to *Crassostrea*). Against this backdrop, evidence from *C. gigantissima* can be examined at that species' type locality and elsewhere.

By original definition (Finch, 1824; Howe, 1937), the type locality of *Crassostrea gigantissima* is Shell Bluff along the Savannah River in Burke County, Georgia (Shell Bluff Landing Ga.-S.C. 1:24000 Quadrangle, 1980 edition, NE 1/4 of NW 1/4). The oysters there occur in late Eocene strata, and Veatch & Stephenson (1911: 245) have provided a general description of the stratigraphy in the upper part of the exposures at Shell Bluff. *Crassostrea gigantissima* is most prominent in a sequence of three superposed oyster-bearing beds, in a partially lithified sand matrix. The oyster remains in each bed "coarsen upward," and the top of each bed is characterized by an intact framework of entire valves and/or articulated shells. Because of its prominence as a ledge-former, the basal bed in this sequence has been the focus of collecting over the years.

The present owners of Shell Bluff have not allowed collecting at the site for a number of years. However, the same sequence of strata can be recognized 6.7 km SSW of the Shell Bluff exposures, as channeled and eroded remnants exposed in roadcuts on the NW side of Ben Hatcher Road (Shell Bluff Land-

ing Ga.-S.C. 1:24000 Quadrangle, 1980 edition, NW 1/4 of SW 1/4), between Fairfield Church and the Newberry Creek bridge. Because of induration, systematic sampling of these exposures has been impossible. But collections do include oysters with chomata on both right (Fig. 1) and left valves; these features are confined to juvenile stages of growth; of fourteen chomata-bearing right valves presently in hand, ten display relict chomata. Thus, *C. gigantissima* in its type beds displays chomata.

Chomata are more prominent in older members of the *Crassostrea soleniscus* lineage. The Cretaceous species *Crassostrea cusseta* Sohl & Kauffman, 1964, is another member of this lineage. Specimens of this latter taxon also display both left and right valve chomata; these features were present in a majority (85%) of the valves available to Sohl & Kauffman (1964: H10) for their original description. Chomata in adult *C. cusseta* do not occur merely as relict chomata; they were produced during more extensive periods of ontogeny and, on the margins of internal cavities range ventrally to mediolateral positions (Sohl & Kauffman, 1964: H10).

The youngest known Atlantic Coastal Plain occurrences of *Crassostrea gigantissima* are in latest Oligocene-earliest Miocene strata of east-central North Carolina (Ward et al., 1978). In systematically collected oysters (Lawrence Belgrade Collection or LBC) from Belgrade, Onslow County, North Carolina (Maysville, North Carolina 1:62500 Quadrangle, 1948 edition, SW 1/4 of NW 1/4; locality described by Lawrence, 1968, 1975), chomata occur infrequently (19 of 141 specimens), like the Eocene occurrences are restricted to juvenile life stages, with one exception (Fig. 2) do occur solely as relict chomata on adult individuals, and appear as slits with raised rims on right valves only (Fig. 2).

In Atlantic Coastal Plain strata, *C. virginica* first appears in units of early Miocene age from New Jersey (Whitfield, 1894; Richards & Harbison, 1942) and the Delmarva Peninsula. Recent construction along U.S. Highway 13, 8.7 km south of Smyrna, Delaware (Dover, Delaware 1:24000 Quadrangle, 1982 edition, NE 1/4 of NW 1/4), uncovered extensive oyster-bearing beds of late early Miocene age (L. W. Ward, personal communication, 1992). Ongoing examination by the writer of a large collection (> 300 individuals of both right and left valves) of these *C. virginica* has not yet recorded the presence of chomata. Thus, Malchus's (1990) notion of the ontogenetic loss of chomata is expressed phylogenetically within this one lineage of crassostreine oysters, suggesting that the presence or absence of these features can be a poor hallmark for the definition of genera within the subfamily Crassostreinae.

Hence, along the Atlantic coast of North America, chomata had disappeared in *Crassostrea* by about 18 million years ago (age from L. W. Ward, personal communication, 1992). There are, however, no reasons to assume that this event was synchronous throughout the entire geographic range of the genus, or even throughout the range of the lineage which includes the type species. *Crassostrea,* with its near-cosmopolitan range, has persisted in a wide variety of environments that could have influenced rates of evolutionary change. In a phylogenetic and temporal sense, the lack of chomata is not a distinguishing character of the genus *Crassostrea.*

Shell Size and Massiveness: The maximum size of shells has been cited as a diagnostic feature of the genera *Pseudoperna, Acutostrea, Indostrea,* and *Konbostrea* (Stenzel, 1971; Chiplonkar & Badve, 1976; Chinzei, 1985; Table 2, bottom, B). This maximum size is largely a function of growth rates and life span or longevity in individual crassostreine oysters, and both of these attributes are dependent upon many extrinsic, environmental factors (Stenzel, 1971: N1027). Without analyzing living oysters of known and differing ages, or without detailed examination and interpretation of periodic growth fabrics (e.g. those of ligamental areas; Stenzel, 1971: N1014-N1016) growth rates and life spans cannot be traced in space and/or time. Such data for crassostreine oysters are meagre at present (Stenzel, 1971: N1014–N1016). Even if patterns of life span are found, they in turn must be interpreted in an acceptable fashion. For example, a preliminary and unpublished analysis by the writer suggests that the early Miocene transition from *C. gigantissima* to *C. virginica* along the Atlantic Coast of North America did involve decreases in maximum life span and a resulting overall smaller size for adult American oysters, but the reason or reasons for these changes remain obscure. Stenzel (1971: N1027) suggested that fossil oysters tend to be larger than still-living ones primarily because of oyster fishing pressures

by humans, but certainly more than a lack of human intervention is responsible for the larger sizes of many fossil crassostreine oysters.

Among shell sizes the extreme elongation of *Konbostrea* warrants special note, because valve heights of over one meter have been cited for this taxon (Chinzei, 1986). But *Crassostrea gigantissima* was itself appropriately named. Heights of over 50 cm for *C. gigantissima* were measured by the writer in the outcrops at Belgrade, North Carolina; valve heights over 66 cm (26 in) have been recorded for other North Carolina occurrences of *C. gigantissima* (Loughlin et al., 1921: 126); and indistinct molds in late Eocene blocky, calcareous clays at Griffins Landing, Burke County, Georgia (Girard, Ga.-S.C. 1:24000 Quadrangle, 1964 edition, NE 1/4 of NW 1/4) suggest even greater heights for this precursor of the American oyster. In summary, maximum adult size is a poor generic designator among crassostreine oysters. The use of maximum size may be useful for species differentiation within a genus, so long as these size differences can be related to reasonable interpretations of life histories and the influence of external, environmental controls.

Significant left valve thickening, largely through chamber formation, has been cited as a common characteristic in taxa of the genus *Striostrea* (Harry, 1985: 150; Table 2, bottom, C). But *Crassostrea gigantissima* displays this same trait. In the LBC collection of *C. gigantissima,* left valve thicknesses range to over 7 cm and estimated valve height to thickness ratios are 3 and lower (compare Harry, 1985: 149–150). Void chambers are prominent in cut sections of thick *C. gigantissima* valves. In some of the LBC individuals, valve thickening occurred without apparent and significant increases in valve height, and this same situation was described by Harry (op. cit.) for *Striostrea.* This attribute, extreme valve thickening, is by no means confined to members of *Striostrea* because it occurs in the lineage including the type species of *Crassostrea.*

Geometries of Umbonal and Ligamental Areas: Freneix (1972: 98–99; 1982) pointed out the crassostreine characters of *Gyrostrea* Mirkamalov, 1963, and removed that genus from the Exogyrinae, where it had been placed by Stenzel (1971: N1125). Spirally coiled growth during postlarval and immature stages, reflected in ligamental and umbonal area outlines, is one important distinction of *Gyrostrea* (Stenzel, op. cit; Table 2, bottom, D). However, *Crassostrea gigantissima* in the LBC materials includes individuals with similar coiling characters, with spiraling ranging to over three-quarters of a volution. Illustrations of *Gyrostrea* (Stenzel, 1971: fig. J99) do not all display preserved coiling, and these figures exhibit only part of the variability that may be found in the systematically collected suite of *C. gigantissima* from Belgrade, North Carolina.

Other differences in the outlines of the ligamental or cardinal area (Table 2, bottom, D) have been cited as diagnostic for numerous crassostreine genera by Chiplonkar & Badve (1979: 445). But these outlines are quite variable in *Crassostrea virginica* (Galtsoff, 1964: figs. 18, 19, 21, 22, 34, 40, 42, 54, 71, 72, 385) and can be influenced by age class and a variety of extrinsic and environmental parameters (Galtsoff, 1964: 16). The LBC specimens of *C. gigantissima* display ostreoid, gyrostreoid, and turkostreoid ligamental area outlines (Siewert, 1972; Malchus, 1990: 77) and also exhibit the variability in overall shell form (plate, triangular, and near-sickle shapes; Malchus, 1990: 89–91) that accompanies this spectrum of dorsal region features. Valve profiles (convex versus concave) strongly depend upon substratum geometry, crowding, and other extrinsic factors and, in the LBC materials, several left valves of *C. gigantissima* display concave profiles; the differences in profiles among genera outlined by Malchus (1990: 95) thus become moot points. In summary, valve form in dorsal and other regions shows considerable within-taxon variability in crassostreine oysters, and these differences cannot be used to separate other proposed genera of the subfamily from *Crassostrea.*

Conical Shell Form: Conical or cup coral-like shell form has been stated to be distinctive of three crassostreine genera—in the external form of older individuals of *Striostrea* and some ecomorphs of *Saccostrea,* and in the internal cavity of gerontic adults of *Konbostrea* (Stenzel, 1971; Harry, 1985; Chinzei, 1986; Table 2, bottom, E). Evolutionary convergence toward a cone-shaped form has been well documented in a number of bivalved organisms, including both molluscs (Yonge, 1962; Perkins, 1969) and brachiopods (Rudwick, 1961; Williams & Rowell,

1965). Crassostreine shell characters involved in this conical form include the development of left valve cupping and umbonal cavities, and the production of prominent chambers, both void and with chalky deposits. As explanations, these conical shell forms have been related to substrata and shell crowding (Stenzel, 1971: N1135; Chinzei, 1986), and the suggestion that adult oysters may continue to accrete their exoskeletons without changing the volume of either their soft tissues or their shell's internal cavity (Stenzel, 1971: N1014).

Conical external form in *Striostrea* and *Saccostrea* is caused by the production of rather prominent left valve umbonal cavities during the development of elongate ligamental areas on that valve. Numerous chambers lie beneath the ligamental area; in dorsoventral sections of left valves, these chambers have a strongly convex/concave outline (Fig. 3; Chinzei, 1982). But these same growth patterns, including the development of striking umbonal cavities, occur in populations of *Crassostrea* (Stenzel, 1971: fig. J101, 1b, 2a); only the degree of development of these features separates *Crassostrea* from *Striostrea* and *Saccostrea.* That such differences help to confer separate generic status is doubtful.

Conical internal cavity form was achieved in a very different fashion in the elongate *Konbostrea.* During elongation, growth of these oysters in dorsal interior regions of the shells was strongly directed toward the opposing valve. This growth pattern involved the production of chalky deposits, strikingly reducing the internal cavity volume in dorsal shell regions; soft tissue connections with the ligament were maintained through a small conical opening; in gerontic forms of *Konbostrea,* the ligament was most certainly dysfunctional (Chinzei, 1986).

These latter growth patterns occur in more elongate specimens of *Crassostrea gigantissima* from the Belgrade collection. In some *C. gigantissima,* ventral displacement of the internal cavity was accomplished by means of the formation of void chambers, but, with their less extreme shell heights, the distinctive conical and dorsal ends of the internal cavity did not develop. Two individuals (LBC-118, 128) apparently maintained a functional ligament by producing a discontinuous, saltated ligamental area during ontogeny. Aspects of growth related to environmentally controlled and strong elongation are thus similar in *Crassostrea* and *Konbostrea,* given the general plasticity of oysters (Gunter, 1954). Only growth details, and the degree of their expression, separate the two genera.

External Ornamentation: The absence of coarse radial ornamentation ("ribs") on left valves was cited by Chiplonkar & Badve (1976, 1979) as diagnostic for their genera *Indostrea, Bosostrea,* and *Cussetostrea* (Table 2, bottom, F). However, left valve ribs may or may not be present in *Crassostrea virginica,* and the development of these valve features in the American oyster is strongly influenced by local environmental factors (Galtsoff, 1964: 18, figs. 4, 15, 21). In the Chesapeake Bay area of North America, for example, commercial oyster fishers have recognized that ribbing is most common in "sand oysters" from intertidal or high subtidal firm bottoms, and in "reef oysters" from intertidal clusters (Kent, 1988). The presence or absence of left valve ribbing is by no means a character worthy of use in distinguishing other crassostreine oysters from *Crassostrea.*

The occurrence of right valve riblets in the prismatic layer of that valve was noted as a marker for *Striostrea* by Stenzel (1971: N1136; Table 2, bottom, F). Although prismatic shell layers may not be commonly preserved in fossil crassostreine oysters, these very riblets appear in prismatic layers on dorsal regions of right valves in *Crassostrea gigantissima* from the LBC materials. In my opinion, one junior synonym of *C. gigantissima* is the taxon *Ostrea alabamiensis* Lea, 1833, described from the Eocene of its namesake state. Right valve radial riblets from juvenile stages have been cited as diagnostic for this taxon (Dall, 1898: 679). Perhaps the Alabama occurrences of this oyster led Stenzel (1971: N1136) to extend the range of *Striostrea* back into the Eocene, because earlier life stages of this latter genus have the typical ostreiform (and not conical) habit. Otherwise Stenzel recognized *Striostrea* as a present-day genus. Regardless of this interpretation, the presence of right valve riblets cannot be used to separate other crassostreine genera from *Crassostrea.*

Muscle Scar Position: Placement of the muscle scar can be influenced by the presence of the promyal passage in crassostreine oysters. This passage is essentially an extension of the epibranchial chamber lying between the mouth and the adductor muscle; the development of the passage may involve

a posterior displacement of the muscle (Nelson, 1938; Gunter, 1954). The passage may be further accommodated by one or more of: increased (relative) shell height, increased left valve cupping, increased development of the left valve umbonal cavity, and migration of adductor muscle attachment to a more ventral position (Gunter, 1950; Sohl & Kauffman, 1964). Thus, it is possible to correlate anatomy with shell features of the Crassostreinae (Sohl & Kauffman, 1964; Stenzel, 1971).

The position of the muscle scar along both dorsoventral and anteroposterior axes has been cited as diagnostic for some genera of crassostreine oysters (Stenzel, 1971; Chiplonkar & Badve, 1979; Table 2, bottom, G). Chiplonkar & Badve (1979) erected the genus *Cussetostrea* (type species *Crassostrea cusseta* Sohl & Kauffman, 1964), and cited a dorsoposterior muscle insertion as one characteristic of their taxon. This diagnosis is based upon a misinterpretation of Sohl & Kauffman's discussion of *Crassostrea cusseta.* Indeed dorsoposterior muscle scar vestiges were cited by Sohl & Kauffman (1964: H10). However, these are interior or within-valve remnants of the oblique track of successive muscle insertion areas (the hypostracum) produced by the oyster during its ontogeny. No discernible muscle scars appear on the internal cavity surfaces of large *Crassostrea cusseta* specimens, in ventroposterior or other positions, and Sohl & Kauffman interpreted this condition to reflect "atrophy of the adductor muscle and subsequent covering of the last formed scar by additional layers of calcite during late maturity and old age" (Sohl & Kauffman, 1964: H11). When formed, the muscle scars of *Crassostrea cusseta* were most likely posterior and medial or ventral in position.

A medial position of the adductor along the dorsoventral axis has been cited for some forms of *Striostrea* (Stenzel, 1971: N1136). But measures of oyster shell form, measures that relate muscle position to that of the soft tissue mass, have not been made (compare Galtsoff, 1964: fig. 42). Numerous *Crassostrea virginica* valves and shells occur along the South Carolina coast (in both present-day and archaeological contexts), the muscle scar placement of which may be qualitatively described as posterior and dorsoventrally medial. Biologically meaningful valve measures (such as a three-dimensional shell midline; Sohl & Kauffman, 1964: H4–H5) might be developed and used to quantify muscle scar placement along both dorsoventral and anteroposterior body axes.

Muscle Scar Geometry: A kidney-shaped muscle scar is cited as diagnostic for the extant genera *Striostrea* and *Saccostrea* by Chiplonkar & Badve (1979: 445; Table 2, bottom, H). However, "reniform" is but one qualitative descriptor for the gibbous, "concave," or nonorbicular muscle scar outlines known in the crassostreine oysters (Stenzel, 1971: N963). These outlines are, in turn, strongly dependent upon overall shell form. In *Crassostrea,* the "typical" scar outlines with abrupt dorsal ends are most obvious in the relatively common and elongate forms. Yet both Galtsoff (1964: fig. 22) and Stenzel (1971: fig. J8) figured subovate/trigonal valves of *C. virginica,* the scars of which have rounded dorsal ends and a distinctly kidney-shaped outline. Galtsoff (1964: fig. 50) illustrated the extreme variability of form within *C. virginica* muscle scars, and Harry (1985: 154) interpreted the muscle scars of all Crassostreinae as reniform in outline. Furthermore, in the fossil record, recognition of scar outline details may be hampered by exfoliation of the surrounding lamellae of the internal shell cavity. Muscle scar outline has no inviolable role in the differentiation of crassostreine genera.

Summary: The fossil genera *Pseudoperna, Acutostrea, Indostrea, Bosostrea, Soleniscostrea, Cussetostrea,* and *Kombostrea* cannot be separated from *Crassostrea,* because all of the tendencies or diagnostic characters proposed by original definers or subsequent and major revisers of these taxa are either environmentally controlled or can be found in *Crassostrea virginica,* the type species of *Crassostrea,* or its immediate ancestor *Crassostrea gigantissima.* Very likely, the reported fossil occurrence of *Striostrea* includes specimens referable to *Crassostrea gigantissima.*

Other Aspects of Living Crassostreine Oysters

Introduction: The genera of living crassostreine oysters (*Crassostrea, Striostrea,* and *Saccostrea*), merit additional comments based upon developmental, genetic, and biogeographic-evolutionary viewpoints. A complete presentation of crassostreine life, biogeography, and evolutionary history is beyond the scope of the present work; the following sections are intended to show some of the problems and prospects in developing

these histories from a taxonomic point of view.

Larval Shells: Larval shells have been accepted as useful in oyster classification since the studies of Ranson (1939, 1943, 1948, 1967) on living species. Ranson recognized only one genus (*Crassostrea*) in the presently acknowledged members of the Crassostreinae, but Dinamani (1976) used studies succeeding those of Ranson (Pascual 1971, 1972; Dinamani, 1973, 1976) to differentiate larvae of *Saccostrea* from those of *Crassostrea* within the subfamily. Critical to this analysis were larval shell form elements in the prodissoconch II (late larval) stage.

Dinamani (1976) noted that larval *Saccostrea* has a hinge margin that remains unmodified throughout larval development and that includes two equal groups of tooth precursors (two to a group) and orthogyrate umbones. Conversely, *Crassostrea* prodissoconch II larvae display inequilateral growth in the hinge margin, with posterior tooth precursors decreasing in prominence and umbones tending toward the opisthogyrate condition. Dinamani (1976: 99) further pointed out that "early larval stages of the *Crassostrea* type have an additional pair of teeth in the left valve."

By contrast Waller (1981: 47–48) chose to accent general similarities in hinge margins among all the Ostreidae during early prodissoconch II stages, and he pointed out that *Crassostrea* starts the prodissoconch II phase with a relatively small size. In comparison to *Ostrea,* prodissoconch II growth in *Crassostrea* contributes more significantly to the overall shape of mature larvae. Because left valve convexity differences appear to begin with prodissoconch II growth, mature *Crassostrea* larvae have left valves that more greatly exceed the right valves in convexity, and left valve umbones that extend farther over the hinge margin, in comparison with their ostreine relatives (Pascual, 1971, 1972; Carriker & Palmer, 1979; Waller, 1981). These same and distinctive geometries also apply to species assigned to *Saccostrea* (Dinamani, 1973, 1976). Antero-posterior differences in growth among the Crassostreinae only develop subsequent to the onset of these other and shared traits. Are such late larval differences significant enough to help confer separate generic status upon *Saccostrea?* The answer to this question need not be affirmative.

Perspectives of Genetics: Studies of genetics provide additional insights into the taxonomy of crassostreine oysters. Hybridization tests have been widely applied to these oysters, with seminal studies by Galtsoff & Smith (1932) and Imai & Sakai (1961). Indeed Stenzel (1971: N1135) argued for separating *Saccostrea* from *Crassostrea* because "species of these two genera cannot be made to crossfertilize each other." More recently Chanley & Dinamani (1980) have questioned the gametic incompatibility of taxa assigned to *Crassostrea* and *Saccostrea* and have noted (Chanley & Dinamani, 1980: 120) that "it is known that sometimes one population of *Crassostrea* will hybridise with other species while another population of the same species will not." These writers, however, did not present evidence to support that last statement. Even if this claim is true, and the results of single hybridization experiments must be evaluated with caution, the failure to achieve partial or complete success in repeated hybridization studies cannot be used to support the recognition or creation of separate crassostreine genera. For reproductive isolation, however achieved, has been one hallmark of biologists' species concept for many decades (Mayr, 1988). The presence of this isolation does not demand the creation of higher, supraspecific taxa; rather, genetic cohesiveness may be viewed as one logical consequence of speciation within genera.

Using crossed immuno-electrophoresis techniques for determining genetic distances, Brock (1990) claimed to substantiate not only the separation of *Ostrea* from the crassostreine oysters, but also the separation of *Saccostrea* from *Crassostrea.* These analyses used pooled tissue homogenates from about 50 individuals from each of six species, two species from each of the three genera. Genetic distances determined were 0.25–0.29 between *Ostrea* and *Crassostrea,* 0.32–0.33 between *Ostrea* and *Saccostrea,* and 0.22–0.26 between *Crassostrea* and *Saccostrea* (Brock, 1990: 61). These data can be interpreted in a variety of ways. First of all, arbitrary values of indices of dissimilarity (or similarity) cannot be used to differentiate taxa at the various hierarchical levels; nor can dissimilarities deemed appropriate (for whatever reason) for use in one taxon necessarily be transferred to another. With the same general range in genetic distances, it can be argued that Brock's data fail to support the separation of these three genera into more than one

family. Also, because of accepted subfamilial biological differences between *Ostrea* and *Crassostrea,* it can be argued conversely, using these same data, that all three genera belong in separate families. Brock (1990: 62) pointed out some of these problems in interpretation. Furthermore, the use of relayed and introduced oysters (Japanese oysters from Oregon, USA) without further explanation does decrease the credibility of Brock's data set. Endemic, natural, and "wild" populations of oysters, collected far from research stations that have a history of handling introduced species, should be actively sought in such studies of genetics. Difficulties in data gathering and interpretation in studies of oyster genetics abound.

Data (Buroker et al., 1979a, b; number of individuals analyzed unpublished) gathered using gel electrophoresis techniques indicate that average heterozygosities for three presumed species assigned to *Saccostrea* (17–19%) fall within the range of six presumed species assigned to *Crassostrea* (6–22%), and that the taxa assigned to *Saccostrea* have greater "between-species" genetic similarities than do taxa assigned to *Crassostrea.* Buroker et al. (1979b: 179) used this observation to suggest, following the fossil record, that "the *Saccostrea* genus is the more recently evolved oyster lineage of the two," but they did not address arguments for or against the separation of *Saccostrea* from *Crassostrea* (see next section of this study). In attempts to determine genetic relatedness, both nuclear and cytoplasmic genetic structures should be considered, and caution should be used in "inferring population genetic structure and gene flow from any single class of genetic markers" (Karl & Avise, 1992: 102).

In the future, expanded taxonomic and geographic studies of crassostreine mitochondrial DNA (Reeb & Avise, 1990; Avise, 1992) should provide meaningful data for the recognition of vicariants and the analysis of paleobiogeographic events. Used in concert with other studies of oyster genetics, such data should help to quell past unnecessary and unfounded speculation about oyster migrations (Stenzel, 1971: N1027; Durve, 1986).

Spatio-Temporal Viewpoints: The genus *Crassostrea,* as recognized by Stenzel (1971), Torigoe (1981), and Harry (1985), has few living species or superspecies (for superspecies concept, see Buroker et al., 1979b). In his synopsis, Harry (1985: 153, 156), assigned only four species to *Crassostrea:* the Atlantic or American oyster, *C. virginica;* the Portuguese oyster, *C. angulata* (Lamarck, 1819) from the eastern North Atlantic; the Japanese oyster, *C. gigas* (Thunberg, 1793), from western Pacific and Indian Oceans; and *C. columbiensis* (Hanley, 1846) from the eastern Pacific. But work predating Harry's has indicated that the Portuguese and Japanese oysters are the same species, based upon likenesses in both adult and larval shells, easily achieved hybridization, and normal meiosis and mitosis in the hybrids (Imai & Sakai, 1961; Menzel, 1974). This finding was foreshadowed by the work of Rutsch (1955), who combined many fossil Cenozoic *Crassostrea* of the Eurasian Tethyan Seaway into the single fossil taxon *C. gryphoides* (Stenzel, 1971: N1081–N1082). The geological record does not demand that the Portuguese and Japanese oysters are separate species, and the fossil record does not promote the notion that the Japanese oyster may have been imported into the eastern Atlantic by humans (compare discussion in Buroker et al., 1979a). That long-separated and present-day populations of the Portuguese and Japanese oysters can hybridize is testament to the conservative nature of the genus *Crassostrea.*

Phylogenetically, the first appearances of still-living *Crassostrea* species are marked by decreases in maximum adult size, and the reasons for these changes are still not clear. The timing of these appearances, and hence rates of evolutionary change, have not been the same throughout the geographic range of *Crassostrea;* reasons for these diachronous changes also need to be explored. By the end of the early Miocene, *Crassostrea* in the northwestern Atlantic Ocean had the essential appearance of *C. virginica,* while members of the lineage to which *C. virginica* belongs, in Panamic regions, still bore chomata on both left and right valves (Woodring, 1982: 612, pl. 94). *Crassostrea* of similar age from North African and/or European Tethyan realms still included extremely elongate and massive-shelled populations, with some individuals bearing chomata (Hoernes & Reuss, 1870; Newton & Smith, 1912). The originally designated type species of the chomata-bearing genus *Saccostrea, S. saccellus* (Dujardin, 1835), = *S. cucullata* (Born, 1778), appeared in Europe during the Miocene (Dollfus & Dautzenberg, 1920), where and when

undoubted *Crassostrea* species bore chomata. These same types of *Crassostrea* may have been present then in other parts of the world, and this taxon in the Neogene (Miocene-Holocene) fossil record needs to be closely re-examined in Sub-Saharan African, northern and southern South American, Indian Oceanic, Asian, and Pacific Oceanic areas.

Interestingly, on mangrove forest or rocky coastlines, living crassostreine oysters of many parts of the world are regularly assigned to *Saccostrea* and *Striostrea* (Stenzel, 1971: N1135; Harry, 1985: 150), whereas similar oysters of the Caribbean and West Indies regions are referred to *Crassostrea* [*C. rhizophorae* (Guilding, 1828), = *C. virginica;* Newball & Carriker (1983), Littlewood & Donovan (1988)]. This difference in assignment, and the lack of living taxa assigned to *Saccostrea* and *Striostrea* in the western North Atlantic, likely reflect the geographically varying rates of evolution within the genus *Crassostrea.*

Regardless of details, *Crassostrea* is the only crassostreine oyster available to yield the taxa presently known by *Striostrea* and *Saccostrea.* The pivotal question becomes: are differences between and among the species significant enough to warrant placement in separate genera? One response is obvious: because the lineage of the type species of *Crassostrea* is so varied in space and time, should anything less be expected of the genus with regard to both soft tissues and exoskeletons? Recognition of but one crassostreine genus would still include, very likely, less than ten living species within *Crassostrea* (Harry, 1985: 149–156). This latter recognition, without subgenera, will help to provide focus upon a number of critical aspects of this taxon.

Paleontologists have continued to define numerous typological species of crassostreine oysters using inadequate samples from the fossil record. If the "expanded" genus has fewer than ten living species, nothing different should be expected at a given "time plane" of the Cenozoic fossil record of this conservative and variable taxon. Our geological knowledge of the crassostreine oysters is limited by fragmentary preservation of strata representing shallow marine and near-shore life environments, but enough productive localities exist (Lawrence, 1968; Laurain, 1980; Moore, 1987; Jimenez et al., 1991) to provide the materials for thorough analysis of variability in form, thus leading to revised synonymies and geographic ranges for fossil species of the genus. Recognition that chomata are a part of the history of *Crassostrea,* and that evolutionary changes in the genus have been diachronous over the face of the earth, are keys to deciphering the geologic history of these oysters. Because chomata are so varied in their occurrence and expression, very large suites of specimens, from controlled intervals of time, are necessary for this geologic work.

Conclusions

In sum, from both paleontologic and neontologic points of view, there are no compelling reasons to recognize more than one genus within the crassostreine oysters. Distinguishing one conservative, plastic taxon may be the only way to focus attention upon the evolutionary history of these oysters.

SYSTEMATICS

OSTREIDAE WILKES, 1810

CRASSOSTREINAE SCARLATO & STAROBOGATOV, 1979

CRASSOSTREA SACCO, 1897

Synonymy

Taxa newly added to the synonymy of Stenzel (1971: N1128) are marked by an asterisk (*).

Crassostrea Sacco, 1897: 15
Gryphaea Fischer, 1886: 927
[non Lamarck, 1801: 398]
**Pseudoperna* Logan, 1899: 95
Crassostrea (Euostrea) Jaworski, 1913: 192
**Saccostrea* Dollfus & Dautzenberg, 1920: 471
Dioeciostrea Orton, 1928: 320
Crasostrea Koch, 1929: 6 (*nom. null.*), *fide* Stenzel, 1971: N1128
Dioeciostraea Thiele, 1934: 814 (*nom. null.*)
**Saxostrea* Iredale, 1936: 269
**Striostrea* Vyalov, 1936: 17
Angustostrea Vyalov, 1936: 18
**Acutostrea* Vyalov, 1936: 18
Grassostrea Vyalov, 1948: 23 (*nom. null.*)
Somalidacna Azzaroli, 1958: 115
Crassotrea Miyake & Noda, 1962: 599 (*nom. null.*)

**Sanostrea* Miyake & Noda, 1962: 599 (*nom. null.*)
**Gyrostrea* Mirkamalov, 1963: 152
**Indostrea* Chiplonkar & Badve, 1976: 245
**Bosostrea* Chiplonkar & Badve, 1978: 106
**Cussetostrea* Chiplonkar & Badve, 1979: 443
**Soleniscostrea* Chiplonkar & Badve, 1979: 444
**Striostrea* (*Parastriostrea*) Harry, 1985: 151
**Konbostrea* Chinzei, 1986: 140
[*non* Seilacher, 1984: 217 (*nom. nud.*)]

Diagnosis

Nonincubatory larvae. Late larval shells with left valve greatly exceeding right valve in convexity; left valve umbone significantly overhanging hinge line; ligament developed far anterior to all tooth precursors.

Adults with right side promyal passage, which may be accommodated by one or more of: a left valve umbonal cavity, left valve cupping, dorsoventral elongation, and posterior and/or ventral displacement of the adductor muscle; none of the latter characters constant in its expression within the genus. Adductor muscle scar nonorbicular. Valve chambers ranging to very prominent. Chalky deposits, when present, nonvesicular under light microscopy.

Remarks

This diagnosis must not be interpreted as merely a "lumping" one, nor will it necessarily lead to the reinstatement of only three genera of living oysters (Ranson, 1943, 1948). Recognition of but one crassostreine genus, with very likely fewer than ten living species, does not require the consideration of separate subgenera, and should help to provide new focus upon the total natural history of this taxon.

This diagnosis also does not ignore or dismiss the many fine studies on oyster biology of the past century. Rather, it restates what oyster biologists have emphasized for many years, the extreme variability of the shells of oysters in the genus *Crassostrea* (Galtsoff, 1964: 27), and extends this concept to soft tissues as well. In understanding phylogenies, nothing has been gained by using the abundance of anatomical data to erect new genera and subgenera of living crassostreine oysters. This revised taxonomy should serve to highlight some of the past and ongoing biological works that are vital to our understanding of the history of these oysters.

One guidepost for future studies, in both present-day and ancient settings, must be a continuing realization that the ultimate and unshakable classification of oysters cannot be constructed by a single investigator (Vyalov, 1948; Stenzel, 1971: N1093). Workers should also remember that "oysters are among the most plastic organisms known" (Gunter, 1954: 134).

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LETTER TO THE EDITOR

CLARIFICATION AND EVALUATION OF TILLIER'S (1989) STYLOMMATOPHORAN MONOGRAPH

Kenneth C. Emberton[1] & Simon Tillier[2]

When KE (Emberton, 1991) performed an independent phylogenetic analysis of 17 of the 111 subfamilies of stylommatophoran land snails previously analyzed by ST (Tillier, 1989), his results differed so greatly that he wrote a detailed critique of ST's monograph (Emberton, unpublished), initiating an exchange between KE and ST that accumulated to about 100 manuscript pages, which we condense into this letter. We are very grateful to Rüdiger Bieler for his useful comments on an earlier draft.

Tillier's (1989) phylogenetic characters, character states, and transformation series (= "morphoclines") were not clearly defined, making his work irreproducable. Here we remedy the problem by defining and illustrating his transformation series (Figs. 1, 2).

1. BM = buccal mass: 1 = spheroidal to ovoidal tending toward cylindrical; 2 = clearly cylindrical (Fig. 1: BM).

2. OC = esophageal crop: 1 = absent; 2 = separated from gastric crop by a distinct portion of the esophagus; 3 = separated from gastric crop by a simple constriction; 4 = as in 3 but extending forward to the nerve ring (Fig. 1: OC).

3. SC = gastric crop: 1 = cylindrical; 2 = median portion inflated; 3 = anterior region inflated; 2′ = funnelform, widening from esophagus to stomach; 0 = unscorable (e.g. semislugs), so eliminated (Fig. 1: SC).

4. PS = gastric pouch: 1 = joining the gastric crop without any constriction, distinctly wider than the gastric crop; 2 = joining the gastric crop without any constriction, slightly wider or no wider than the gastric crop; 2′ = separated from gastric crop by a constriction, distinctly wider than the gastric crop (Fig. 1: PS).

5. IL = intestine length (relative to the combined lengths of the gastric crop and stomach): 1 = intestinal loops reaching a level between the distal limit of gastric pouch and the middle of gastric crop; 2 = intestine shorter, but intestinal loops distinct; 3 = intestinal loops reduced to an almost flat sigmoid; 2′ = intestinal loops long, reaching proximally at least the level of the distal limit of the gastric pouch (Fig. 1: IL).

6. LR = ratio of kidney length to lung length: 1 = 0.45–0.7; 2′ = 0.7–1.0; 2 = 0.36–0.45; 3 = 0.25–0.36; 4 = 0.0–0.25; with semislugs and slugs not scored at all (Fig. 1: LR).

7. UR = degree of closure of the ureter: 1 = no closed retrograde ureter; 2 = closed ureter reaching at most lung top; 3 = ureteric tube reaching a point between lung top and pneumostome; 4 = ureteric tube reaching the pneumostome (full sigmurethry) (Fig. 1: UR).

8. RR = kidney internal morphology: 1 = either two distinct regions (the distal one usually lacking lamellae) or three distinct regions (the median one either lacking lamellae or with lamellae different in appearance from those in the proximal region); 2 = kidney homogeneous in internal morphology, with lamellae reaching the distal region and the level of the kidney pore (Fig. 1: RR).

9. CC = length of cerebral commissure: 1 = greater than 1.1 × right cerebral ganglion width; 2 = between 1.1 and 0.9 × right cerebral ganglion width; 3 = less than 0.9 × right cerebral ganglion width (Fig. 2: CC).

10. CPD = length of the right cerebro-pedal

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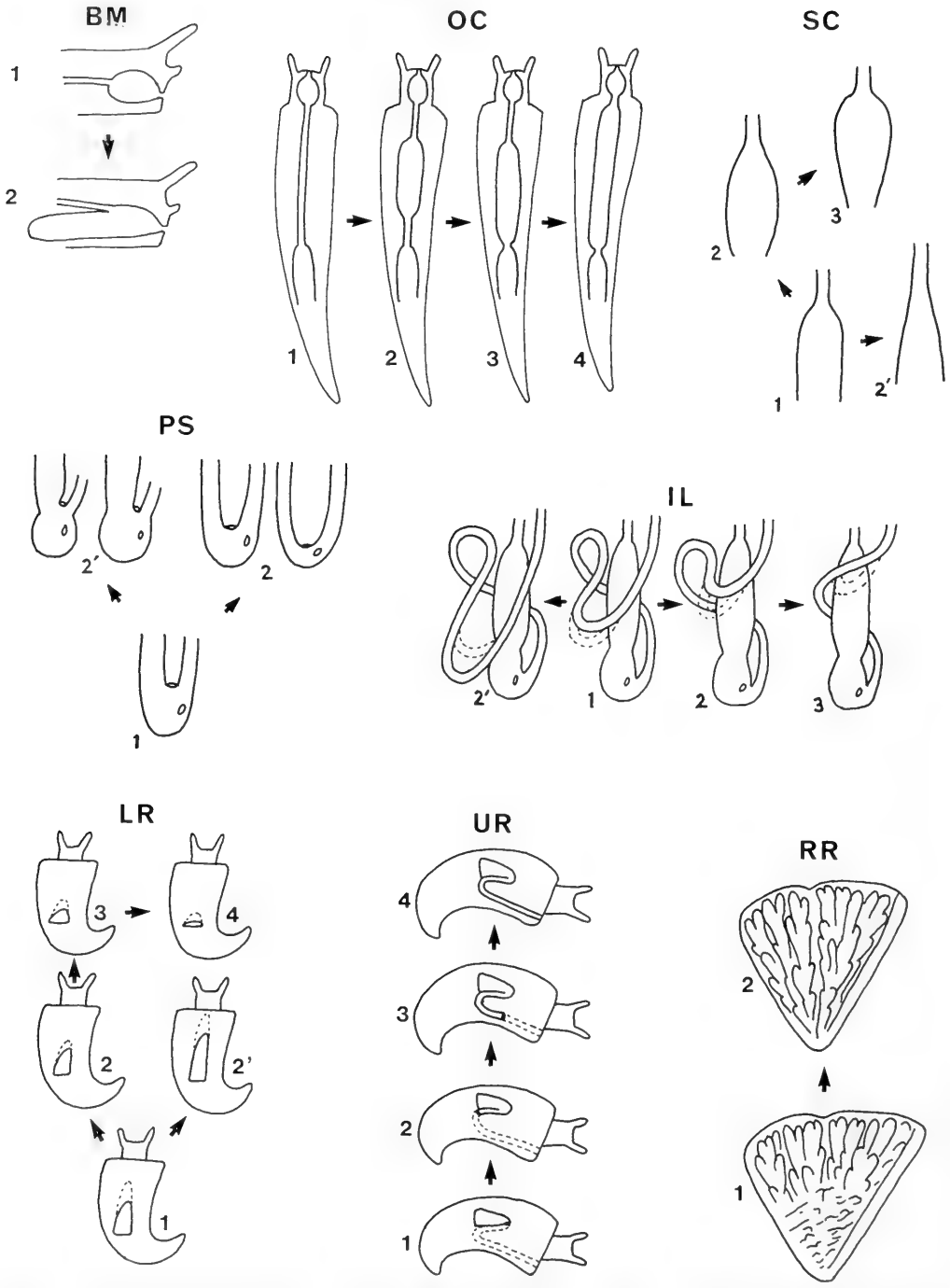

FIG. 1. Diagrammatic illustrations of Tillier's (1989: appendix E) cladistic characters and their character-state transformations. See text for names and definitions.

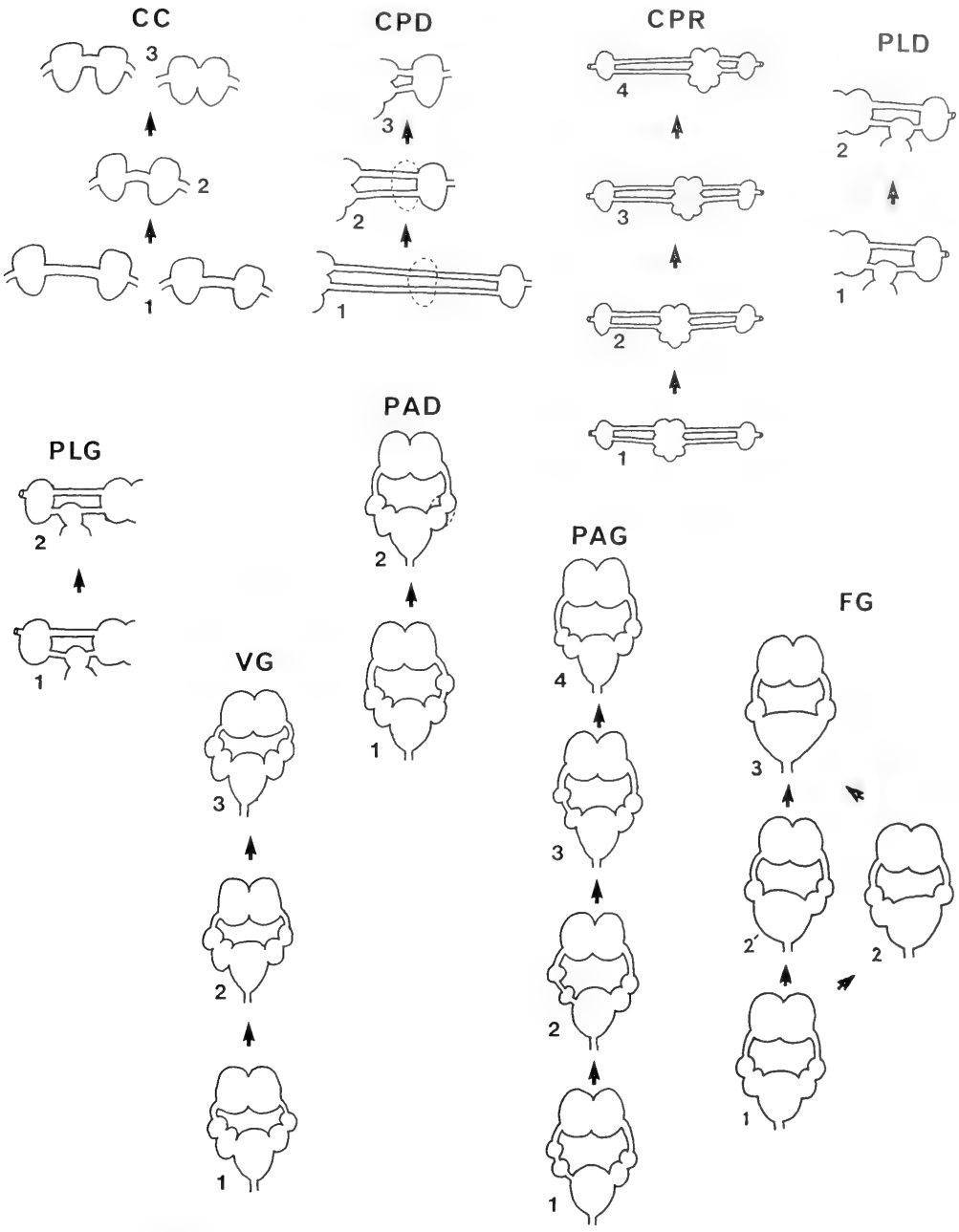

FIG. 2. Diagrammatic illustrations (cont.) of Tillier's (1989: appendix E) cladistic characters and their character-state transformations. See text for names and definitions.

connective: 1 = longer than twice the width of the right cerebral ganglion; 2 = between one and two times right cerebral ganglion width; 3 = shorter than right cerebral ganglion width (Fig. 2: CPD).

11. CPR = ratio between the lengths of the

cerebro-pedal connectives (left/right): 1 = less than 0.9; 2 = from 0.9 to 1.1; 3 = from 1.1 to 1.5; 4 = from 1.5 to 2.5 (Fig. 2: CPR).

12. PLD = position of the right pleural ganglion: 1 = closer to the pedal ganglion than to the cerebral ganglion (hypoathroid); 2 = closer to the cerebral ganglion than to the pedal ganglion (epiathroid); 0 = unscorable, so eliminated (Fig. 2: PLD).

13. PLG = position of the right pleural ganglion: 1 = closer to the pedal ganglion than to the cerebral ganglion (hypoathroid); 2 = closer to the cerebral ganglion than to the pedal ganglion (epiathroid); 0 = unscorable, so eliminated (Fig. 2).

14. VG = position of the center of mass of the visceral ganglion relative to the median plane of the pedal ganglia: 1 = on the right side; 2 = in the middle; 3 = on the left side (Fig. 2: VG).

15. PAD = right parietal and pleural ganglia: 1 = separate; 2 = in contact or fused (Fig. 2: PAD).

16. PAG = position of the left parietal ganglion: 1 = in contact with left pleural, or closer to the left pleural than to the visceral, and separated from both by a distinct connective; 2 = closer to the visceral than to the left pleural, and separated from both by a distinct connective; 3 = in contact with the visceral ganglion alone, and separated from the left pleural by a distinct connective; 4 = in contact or fused with both left pleural and visceral ganglia (Fig. 2: PAG).

17. FG = fusion of the visceral ganglion: 1 = none; 2 = with the right parietal ganglion; 3 = with both parietal ganglia; 2′ = with the left parietal ganglion (Fig. 2: FG).

ST stresses that these character states were not always the same as those used in his "factor analyses" (Tillier, 1989: text-figs. 5–7, 10–18). Character states were first tentatively defined for correspondence analysis (see below), evaluated, and then re-defined and re-scored for phylogenetics. For example, when a character state such as gastric crop shape (SC) in semislugs could not be placed within a transition series, it was redefined as state 0 and eliminated from phylogenetic analysis.

ST's "factor analysis" is not the statistical method known to most American workers as factor analysis, but the method of Benzécri (1973: "analyse factorielle des correspondances") that is better translated as "correspondence analysis." Correspondence analysis, unlike factor analysis, requires no multivariate-normal assumption (Fénelon, 1981; Jambu & Lebeaux, 1979).

KE disagrees with most of ST's character-state choices because they (a) oversimplify a complex character into a single measurement, ratio, or quality (BM, LR, RR, CC, CPD, CPR, PLD, PLG); (b) apply arbitrary cutpoints to continuous variation (BM, IL, LR, UR, CC, CPD, CPR, PLD, PLG); and/or (c) include possible artifacts of fixation, preservation, and dissection (food bolus in OC, SC, PS; stretching in CC, CPD, CPR, VG [Emberton, 1989: fig. 4]).

KE and ST agree that ganglionic fusion (FG) is an important but difficult-to-score character. ST rechecked his dissections of *Anguispira, Sagda* and *Thysanophora* and found that he had scored them incorrectly for ganglionic fusion: correct scorings are as in Emberton (1991). KE also scored *Acavus, Bradybaena,* and *Polygyrella* differently from ST, who did not recheck these genera. KE disagrees with ST's opinion that ganglionic contact (short of fusion) is a reliable character.

KE advocates use of structurally complex characters divisible into discrete, qualitative states. ST rebuts that this is impractical for soft-part molluscan anatomy, and that all the transformations KE (Emberton, 1991) used, with the possible exceptions of his characters 4 and 5, would prove to be continuous if more taxa were included. KE doubts that his characters 2–5 and 8–18 will prove continuous, but agrees that 1, 6, and 7 may; KE counters that ST made many of his (ST's) characters artificially continuous by reducing them to measurements and ratios.

Users of ST's anatomical figures are cautioned that sinistral species (with all organs right-to-left reversed) are not indicated as such in the captions (e.g. Tillier, 1989: figs. 111, 484, 512).

As discussed more recently by Tillier & Ponder (1992), the Otinidae were used by ST as the stylommatophoran sister group because they share with the Stylommatophora (a) monotremy, (b) kidney not surrounded by lung, and (c) five ganglia in the ventral chain. This position is not accepted by Nordsieck (1992), who proposed the Ellobiidae as the sister-group of the Stylommatophora.

ST's (Tillier, 1989) "phylogenetic analysis" was performed in 1984 (Tillier, 1985), using the then unpublished algorithm of Delattre (1988), which does not permit reversals,

which may not find the most parsimonious tree(s), and which KE believes is a phenetic rather than cladistic method. Obviously, the more recent availability of more efficient cladistic algorithms has made this part of ST's work obsolete; reanalysis using Hennig86 (Farris, 1988) yields a very different topology (Richard Lamb, personal communication). KE contends that reanalysing ST's tabulated data is unproductive because of numerous defects in the conception and scoring of characters.

The Orthurethra/non-Orthurethra split proposed by Pilsbry (1900) remains the only aspect of stylommatophoran phylogeny supported by ST's morphological data. The division of the order Stylommatophora proposed by ST (Tillier, 1989) into two suborders, Brachynephra and Dolichonephra, was submerged by Nordsieck (1992) correctly in KE's opinion, although ST believes that additional data may support these taxa.

The multiplication of phylogenetic hypotheses in the past 15 years, as summarized by Bieler (1993), shows in our opinion that: (a) monophyly remains undemonstrated for most families and suprafamilial taxa; (b) there are high levels of homoplasy in all known anatomical characters; (c) any worthwhile further morphological study should include numerous taxa and characters, should include careful character analyses, and should clearly define and illustrate all character states and suggested transformations; (d) histological sections will be required to resolve some potentially important characters, such as fusion among ventral-chain ganglia, kidney internal morphology, and the fertilization pouch-seminal receptacle complex. Other anatomical characters worth comparing may be the ureteric interramus and nearby structures, the position of the proximal hermaphroditic duct, the fusion of the free retractor muscles, and genital accessory organs. Molecular characters may prove useful, as shown by Emberton et al. (1990) and Tillier et al. (1992, and in press).

Thus despite over a century of work, only two suprafamilial clades of the Stylommatophora, Orthurethra and Sigmurethra, may be resolved, although recent molecular studies by ST cause him to question even the monophyly of the Sigmurethra. Resolution of stylommatophoran higher phylogeny remains a tremendous but hopefully not impossible challenge.

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AWARDS FOR STUDY AT
The Academy of Natural Sciences of Philadelphia

The Academy of Natural Sciences of Philadelphia, through its Jessup and McHenry funds, makes available each year a limited number of awards to support students pursuing natural history studies at the Academy. These awards are primarily intended to assist predoctoral and immediate postdoctoral students. Awards usually include a stipend to help defray living expenses, and support for travel to and from the Academy. Application deadlines are 1 March and 1 October each year. Further information may be obtained by writing to: Chairman, Jessup-McHenry Award Committee, Academy of Natural Sciences of Philadelphia, 1900 Benjamin Franklin Parkway, Philadelphia, Pennsylvania 19103-1195, U.S.A.

INSTRUCTIONS FOR AUTHORS

1. MALACOLOGIA publishes original research on the Mollusca that is of high quality and of broad international interest. Papers combining synthesis with innovation are particularly desired. While publishing symposia from time to time, MALACOLOGIA encourages submission of single manuscripts on diverse topics. Papers of local geographical or systematic interest should be submitted elsewhere, as should papers whose primary thrust is physiology or biochemistry. Nearly all branches of malacology are represented on the pages of MALACOLOGIA.

2. Manuscripts submitted for publication are received with the tacit understanding that they have not been submitted or published elsewhere in whole or in part.

3. Manuscripts may be in English, French, German or Spanish. Papers in languages other than English must include a translation of the Abstract in English. Authors desiring to have their abstracts published in other languages must provide the translations (complete with main titles). Include all foreign accents. Both American and British spellings are allowed.

4. Unless indicated otherwise below, contributors should follow the recommendations in the *Council of Biology Editors (CBE) Style Manual* (ed. 5, 1983) available for U.S. $24.00 from CBE, 9650 Rockville Pike, Bethesda, MD 20814, U.S.A.

5. Be brief.

6. Manuscripts must be typed on one side of good quality white paper, double-spaced throughout (including the references, tables and figure captions), and with ample margins. Tables and figure captions should be typed on separate pages and put at the end of the manuscript. Make the hierarchy of headings within the text simple and consistent. Avoid internal page references (which have to be added in page proof).

7. Choose a running title (a shortened version of the main title) of fewer than 50 letters and spaces.

8. Provide a concise and informative Abstract summarizing not only contents but results. A separate summary generally is superfluous.

9. Supply between five and eight key (topic) words to go at the end of the Abstract.

10. Use the metric system throughout. Micron should be abbreviated μm.

11. Illustrations are printed either in one column or the full width of a page of the journal, so plan accordingly. The maximum size of a printed figure is 13.5 × 20.0 cm (preferably not as tall as this so that the caption does not have to be on the opposite page).

12. Drawings and lettering must be dark black on white, blue tracing, or blue-lined paper. Lines, stippling, letters and numbers should be thick enough to allow reduction by ½ or ⅓. Letters and numbers should be at least 3 mm high after reduction. Several drawings or photographs may be grouped together to fit a page. Photographs are to be high contrast. High contrast is especially important for histological photographs.

13. All illustrations are to be numbered sequentially as figures (not grouped as plates or as lettered subseries), and are to be arranged as closely as possible to the order in which they are first cited in the text. Each figure must be cited in the text.

14. Scale lines are required for all nondiagrammatic figures, and should be convenient lengths (e.g., "200 μm," not "163 μm"). Magnifications in captions are not acceptable.

15. All illustrations should be mounted, numbered, labeled or lettered, i.e. ready for the printer.

16. A caption should summarize what is shown in an illustration, and should not duplicate information given in the text. Each lettered abbreviation labeling an individual feature in a figure must either be explained in each caption (listed alphabetically), or be grouped in one alphabetic sequence after the Methods section. Use the latter method if many abbreviations are repeated on different figures.

17. Tables are to be used sparingly, and vertical lines not at all.

18. References cited in the text must be in the Literature Cited section and *vice versa*. Follow a recent issue of MALACOLOGIA for bibliographic style, noting especially that serials are cited unabbreviated. Supply pagination for books. Supply information on plates, etc., only if they are not included in the pagination.

19. In systematic papers, synonymies should not give complete citations but should relate by author, date and page to the Literature Cited section.

20. For systematic papers, all new type-specimens must be deposited in museums where they may be studied by other scientists. Likewise MALACOLOGIA requires that voucher specimens upon which a paper is based be deposited in a museum where they may eventually be reidentified.

21. Submit each manuscript in triplicate. The second and third copies can be reproductions.

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24. When an article is 10 or more printed pages long, MALACOLOGIA requests that an author pay part of the publication costs if grant or institutional support is available.

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