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ARE PARTICULAR CHROMOSOMES SEX DETERMINANTS?

THOS. H. MONTGOMERY, JR.

The past decade has witnessed greatly renewed interest in the problems of sex determination, due very largely to the study of hybridization and the broad application of the results. Concurrently the investigation of the germ cells has increased in amount in a geometrical ratio; never before have these cells so fully engaged the thoughts of biologists, and our knowledge of the complex chromosomal activities has increased to an extent unpredicted.

In particular have multiplied investigations of the modified chromosomes, now described in animals for the spermatogenesis in insects, araneads, myriopods, copepoda, Sagitta and birds: and for the oogenesis in echinoderms, the cat, and possibly some insects (*Pyrrhocoris*, *Gryllus*). Following the terminology given by me in 1906, these may be collectively named allosomes, a term more convenient than my earlier one of heterochromosomes, in contrast to the unmodified chromosomes or autosomes. They have received a great variety of names: accessory, special, lagging, heterotropic, sex chromosomes; idiochromosomes, microchromosomes, diplosomes, gonochromosomes, chromatin nucleoli, etc. Any body within a nucleus that stains like chromatin should not, however, be considered an allosome until its chromosomal nature be ascertained.

Within the past eight years an hypothesis has arisen ascribing sex-determining properties to these allosomes, and my object is to treat this hypothesis first historically, and second critically.

A. The Hypothesis.

The first statement of the hypothesis is due to McClung in 1902, after investigation of the unpaired accessory chromosome in the spermatogenesis of Orthoptera — that kind of modified chromosome named by me in 1906 the monosome. McClung's conception of the function exercised by the accessory chromosome is "that it is the bearer of those qualities which pertain to the male organism, primary among which is the faculty of producing sex cells that have the form of spermatozoa." He recognized also that there must be selective fertilization, that to the ovum "come the two forms of spermatozoa from which selection is made in response to environmental necessities." At that time nothing was known of the maternal chromosomal number, so that it was natural for McClung to reason that the monosome was a paternal chromosome not represented in the female.

In the same year Sutton (1902) described for *Brachystola* that "twenty-three is the number of chromosomes in the male cells, while twenty-two is the number I have found in the female cells, and thus we seem to find a confirmation of McClung's suggestion that the accessory chromosome is in some way concerned in the determination of sex." Subsequent studies have shown that Sutton was wrong in his count of the oogonial chromosomes.

Then Stevens (1905) found in *Tenebrio* "that in both somatic and germ cells of the two sexes there is a difference not in the number of chromatin elements, but in the size of one, which is very small in the male and of the same size as the other nineteen in the female. . . . The small chromosome itself may not be a sex determinant, but the conditions in *Tenebrio* indicate that sex may in some cases be determined by a difference in the amount or quality of the chromatin in different spermatozoa." In 1906 she wrote: "The scheme also assumes either selective fertilization, or, what amounts to the same thing, infertility of gametic unions where like sex chromosomes are present"; and in 1909*a*: "The only other alternative in these insects seems to be that sex is already determined in the egg before fertilization, either as a matter of dominance, or as a result of maturation, and that fertilization is selective . . . but any such general application is premature until adequate evidence is at hand to prove that

the sex character is represented in the chromosomes." Yet in another paper (1909*b*) that appeared simultaneously, Stevens writes: "As to the fact that the lagging chromosome of the aphids is a heterochromosome intimately connected with the phenomenon of sex determination, the present investigation of the male germ cells, I think, leaves no doubt."

Independently of Miss Stevens, and simultaneously, Wilson found that where there is a single monosome in the male, it is represented by a pair in the female; and where a large and small idiochromosome in the former, there is a pair of large ones in the latter. In his first two studies (1905*a*, *b*) he expressed himself guardedly as to such chromosomes being associated with sexual phenomena. In his third study (1906) he gives a much fuller discussion, and it is this treatment more than any other, that has aroused general interest in the subject. He mentions as one alternative that merely quantitative difference in amount of the chromatin may be the determining factor, but he criticises this for the reason that in *Nezara* the idiochromosomes are of equal size in both sexes, while a series of intergradations are known between such a condition and the one where these elements are dissimilar in size in the sexes. Therefore he maintains the alternative view, that the allosomes have qualitative differences that are sex-determining, with Mendelian dominance, and with selective fertilization. "The general interpretation . . . must include the assumption that there are two kinds of eggs (presumably in approximately equal numbers) that contain respectively the male and the female-determinant, and that the former are fertilized only by spermatozoa that lack the heterotropic chromosome (*i. e.*, the male determinant) and *vice versa*. . . . Such a selective fertilization is therefore a *sine qua non* of the assumption that the heterotropic chromosome is a specific sex-determinant." In this argument Wilson makes use of Castle's (1903) hypothesis that sex follows the rule of Mendelian segregation and dominance. To this view he adheres also in his fourth and fifth studies (1909*a*, *b*). But in his last paper (1909*c*) he opposes the Mendelian interpretation, because selective fertilization is improbable. He brings as another objection the case of the bee; here the egg after two maturation divisions

forms a male if not fertilized, a female if fertilized; under the hypothesis the female tendency should be derived from the spermatozoon—"a *reductio ad absurdum;* for the male is derived from an unfertilized egg which has by the hypothesis eliminated the female tendency."

Nowlin (1906) and Boring (1907) analyzed the phenomena of the allosomes in Hemiptera and Coleoptera respectively, and Jordan (1908) in an orthopteran, all comparing chromosomal numbers in the female and male cells, and all inclined to regard the allosomes as sex-determinants. Morrill (1909) found that in *Protenor* and other species all the cleavage cells of one individual have either thirteen or fourteen chromosomes, *i. e.*, all either a single allosome or a pair, conformable with Wilson's results on the germ cells.[1]

Payne (1909) has accepted Wilson's views of a qualitative sex-determination by allosomes; while von Baehr (1909), in discussing the subject at length, is inclined towards the quantitative explanation. Wallace (1909) concluded that in *Agelena* some spermatozoa have two allosomes, others have none, and argued that a male would result when an ovum is fertilized by a sperm with these two elements; it should be noted, however, that the different describers of aranead spermatogenesis have reached quite conflicting results.[2]

Baltzer (1909) on echinoids, drew attention to the occurrence of allosomes (idiochrosomes) in the female line, though he studied the chrosomomes only in the pronuclei and cleavage cells, not in the growth period of the oöcytes. "We distinguish accordingly two types of eggs: some with and some without an unpaired element. The chromosome number is in both cases eighteen. Therefore, we must conclude that the unpaired chromosome in the egg type where it is wanting is represented by a rod-shaped element. The sperms—always with eighteen elements—are all alike. According to the discoveries on insects, it is not improbable that the determination of sex, which would lie with the female, is connected with this difference of the egg nuclei."

[1] Some writers have denominated this case "*Wilson's Protenor-type*," evidently ignorant that the spermatogenesis of this form was rather fully described by me (1901) some years before Wilson published his studies.

[2] Cf. the papers of Wagner, Berry, Bösenberg and myself.

Boveri (1909*a*) refers to Baltzer's work as indicative of sex-determination by particular chromosomes (the short hook-shaped ones). But in opposition to Wilson's explanation, he does not believe that one chromosome has a male and the other a female tendency, but that they differ only in activity: the larger allosome would give to a cell a larger power of assimilation, and such a view "would perhaps be qualified to serve as a basis for a general theory of sex-determination." Boring (1909) described the occurrence in the eggs of *Ascaris megalocephala* of a fifth, small chromosome element, but was unable to decide whether this is "a chromosome unit in itself, or a fragment of one of the long chromosomes," and considered it merely possible that it might be sex-determining. Boveri (1909*b*) holds this occasional structure to be undoubtedly sex-determining, a chromosome unit that is in most cases attached to the end of one of the others. At the same time he reports the occurrence of a monosome in the spermatogenesis of *Heterakis*, and concludes, in the sense of Wilson: "Fertilization of an egg by a sperm with five chromosomes leads to development of a female, by a sperm with four elements to production of a male."[1]

Finally, in the last paper on this subject, Edwards (1910) describes allosomes in the spermatogenesis of certain individuals of *Ascaris megalocephala*, and does not hesitate to call them sex-determinants.

B. Previous Criticisms of the Hypothesis.

In the preceding lines we have endeavored to state, in all brevity, the nature of the arguments advanced to prove that particular allosomes produce one sex or the other by their presence or absence, whether by qualitative or quantitative differences. Now we may consider certain objections that have been raised to such interpretations.

I have remained skeptical with regard to these hypotheses, though I have described many cases of allosomes in a succession

[1]On p. 135, Boveri refers to "his" discovery in 1904 that "the tetrads of the first oöcyte division of *Ascaris meg. bivalens* can consist of two shorter and two longer rodlets," whereas this was (1904) particularly described and figured by me earlier in the same year.

of papers, and have expressed myself only once on the matter, in 1906, in considering Wilson's contention which I regarded "a very plausible conclusion, but there are in particular two phenomena which must be explained before it can be accepted. One is, how an allosome becomes lost in the spermatogenesis; and the other is, how the allosomes introduced by the spermatozoön into the ovum behave during the ovogenetic cycle; on both of these questions we know as yet practically nothing."

Gross (1904) objected to the hypothesis of McClung, (1) that it is not proven that accessory chromosomes are absent in females, and (2) the case of the bee, where males develop from unfertilized eggs. He also believed that the spermatozoa with monosomes may be incapable of fertilization; but failed to note that such supposition could not be applied to sperm with idiochromosomes.

Foot and Strobell (1909) urged that the theory of the individuality of the chromosomes is not proven—today, a decided minority view. They also held that the allosomes of *Euschistus* are not chromosomes at all, and are variable in number, which is in direct opposition to the discoveries of Wilson and myself. "In the case of *Euschistus* we are told that the larger of the two chromatin nucleoli of the spermatocyte is the homologue of the accessory chromosome of other forms, and if this interpretation is correct we may expect to find a large bivalent or two univalent chromatin nucleoli in the growing oöcytes." But they find no such bodies in oöcytes, and therefore conclude that the chromatin nucleoli of the male are never transmitted to the egg, are not chromosomes at all, and hence cannot be sex-determining; this objection to the Wilson-Stevens theory is inadmissible.

An explanation suggests itself to me why allosomes, which all evidence leads us to believe must be transmitted to eggs by fertilization, act in a different way in the oögenetic cycle. That is, in spermatogenesis the single monosome, or the pair of unequal idiochromosomes, behave differently from the other chromosomes, remaining dense and compact in the growth period of the spermatocytes, probably because they are there unpaired (monosome) or of unequal size (idiochromosome), while all the other chromosomes are paired, and the two of each pair seemingly alike. In the growth period of the oocytes, on the contrary, the

allosomes of the spermatocytes seem to be represented by a pair of elements similar in all respects to each other; there is no dissimilarity of the pair, hence no conspicuous behavior different from that of the other chromosomes (autosomes). In other words, it is the singleness of the elements (monosomes), or their disparity in size and activity (idiochromosomes), that may be a reason why the allosomes behave so peculiarly in spermatogenesis. For these various considerations the arguments of Foot and Strobell against the hypothesis are not valid, though these investigators are quite right in seeing the necessity of comparing the oögenesis.

Buchner (1909) has entered other arguments against the determination of sex by allosomes, an hypothesis that he wholly rejects. He refers to their limited occurrence, which shows they could not be universal sex-determinants. Then to the occurrence of an accessory chromosome in the oögenesis, paralleling that in the spermatogenesis of *Gryllus*, he calls particular attention, insisting that it is necessary for the Wilson-Stevens theory that sperm-cells alone should have allosomes. "In *Gryllus* there can be no talk of a sex-determining function, and thereby naturally also not in the other animals with accessory chromosomes."[1]

Gutherz (1909) has, however, combated Buchner's opinion that the chromatic body in the oöcytes of *Gryllus* is a chromosome, and shows that it differs from such in many details. He also finds that "the diploid chromosome group of the male numbers 21, that of the female 22 chromosomes." "The doctrine

[1]There are a couple of points in which Buchner seems to be in error. He states (p. 409) that Goldschmidt had argued in 1904 that the allosomes might represent "trophic chromatin," and accuses me of having in 1906 overlooked Goldschmidt's priority. As a matter of fact, I wrote in 1901: "Thus it might be that in the insects the chromatic nucleoli are those chromosomes which either exert a greater metabolic activity than the other chromosomes, or which carry out some special kind of metabolism." It was Goldschmidt who had overlooked my earlier statement of this view. Then on p. 415, Buchner concludes that "the accessory chromosome (monosome) is no unit body, as has been generally assumed up to this time, but a bivalent with non-equivalent components." But in 1901, and again in 1905, I argued that the larger monosomes of sperm cells may be bivalent elements, the pair that is separate in the oögenesis represented by a pair in fusion in spermatogenesis.

of the connection between heterochromosomes and sex-determination is accordingly not disturbed by this discovery."

Morgan (1909, 1907) classes the theories of sex determination by chromosomes as qualitative and quantitative, and inclines to the latter view—he being the first to take this stand positively. By a quantitative interpretation he does "not mean that the female is simply male plus something else, a view recently advanced by Castle, but that male and female are two alternate possibilities of the living material, which possibility is realized depending on quantitative factors. . . . The gametes are not, therefore, male and female, but contain certain factors which, when combined, give rise, in an epigenetic fashion, to one or the other alternative." In the phylloxerans, the "loss of certain chromosomes from the male egg appears to follow, not to precede the size relation. . . . But there is nothing in these facts that shows that the effects are directly quantitative rather than that observable quantitative differences accompany, or follow in some cases, more profound changes." He considers as the most serious objection to the qualitative interpretation "that although the hypothesis is ostensibly based on the presence of certain chromosomes which are assumed to be male and female determining respectively, yet to these chromosomes, which are to all appearances identical, are ascribed exactly opposite functions." Morgan's whole attitude is rather hostile to the view that particular chromosomes are sex-determinants, and his arguments against the view are the most cogent yet presented.

C. Further Criticisms of the Hypothesis.

In a previous treatment (1906*a*) of the phenomenon of sexuality, I was led to define it (p. 85) as "essentially the condition of difference obtaining between conjugating individuals. . . . Because conjugation is a process distinct from reproduction, sexuality, being intimately associated with conjugation, has no primary connection with reproduction. . . . The genesis of sexuality has been this: that out of a state where all individuals were equally capable of reproduction a condition of division of labor has ensued, inducing morphological and chemical differences, between individuals capable of reproduction and conjugation and other individuals

capable of reproduction and conjugation alone. This holds true in the Metazoa, both for the germ cells and for the persons, and the male is characterized by his power to conjugate or fertilize, the female by her power to reproduce. A microgamete in the Protozoa, or a spermatozoön or male person in the Metazoa, is an individual that has lost the power of reproduction in becoming specialized for the act of conjugation. Sexuality is then the state of occurrence of dissimilar conjugating individuals, and the essential point in this dissimilarity is that only one kind of these individuals has the power to reproduce. This simple interpretation was entirely overlooked by Geddes and Thomson in their theory of 'The Evolution of Sex.' ".

The germ cells are then not without sex, as Morgan would have us believe, but have an actual *sexuality* with respect to each other, an ovum being female and a spermatozoön male; as well as a *prospective* sexuality with regard to the kind of individual they may engender. We are here concerned with the question of the determination by particular chromosomes of prospective sexuality.

Further, a hermaphrodite is bisexual, and its egg therefore prospectively bisexual, engendering both kinds of gametes. This indicates that an egg may contain potentially the characters of both sexes, or better stated, that both states of sex may arise from the same egg. This may also be true for species that are not hermaphrodite, for a female individual frequently shows certain male characteristics, and a male certain female qualities, even if in a more or less latent condition. These conditions indicate that an egg does not contain prospectively one sexual state to the exclusion of the other, but rather that maleness and femaleness are closely associated phenomena that may interchange within the same individual; a possibility suggested by Morgan (1909).

Bearing these ideas of the value of sex in mind, the following main objections may be made to the hypothesis that particular allosomes act as sex determinants:

1. While the phenomena appear to admit of a simple explanation in cases where there are only a pair of idiochromosomes, or a single monosome, in the spermatogenesis, often the conditions

of the allosomes are so much more complex than this, allowing so many different chromosomal combinations in the spermatozoa, that the interpretation of what spermatozoa are male-producing and what are female-producing becomes very difficult. Attention may be drawn, for example, to a case in the Hemiptera described by me (1901, 1906*b*). In spermatocytes of *Calocoris rapidus* there are: "twelve autosomes that divide in both mitoses, two diplosomes that do likewise (therefore are probably also bivalent), a smaller monosome that does not divide in the first but does divide in the second mitosis, and a larger monosome that divides in the first but not in the second mitosis." Other complex associations of allosomes have been described by McClung and Payne. Were the allosomes sex-determinants, we would have to conclude that in certain species a considerable number of the chromosomes subserve this end, which would be allotting an undue amount of the nuclear material to this purpose.

2. In all plants, with the exception of one (*Salomonia*) described by Cardiff (1906), and in may animals, no allosomes are known, yet these species have sexuality. It is probable that such structures will be found in certain cases where they have may been overlooked; yet they are apparently absent in some cases where special search has been made for them; accordingly, at the most they can be sex-determinants in only a limited number of cases.

3. In certain species there is the phenomenon of two sizes of eggs, some larger that produce females, others smaller and male-producing. This is known for the Phylloxerans, Rotatoria and *Dinophilus apatris;* another case has been described for an acarine by Reuter (1907); and I have shown (1907) that there are two sizes of eggs in the aranead *Theridium*, though I did not raise these eggs to determine their prospective sex values. These two kinds of eggs may be produced by the same individual, or (Rotatoria, Punnett, 1906) by different individuals. These eggs become distinguishable in the growth period, and for the Phylloxerans Morgan (1909) has shown that the egg is "sexually determined" before the formation of the polar bodies. Malsen (1906) held for *Dinophilus apatris* that the "difference between male and female eggs apparently lies chiefly in the greater or less number of fusing ovogonia"; but his brief description and few figures do

not prove this point. But however these differences arise they are clearly present early in the growth period, which is strong evidence that they cannot be produced by any sorting of allosomes in fertilization. And it is quite possible, as Beard has reasoned, that a distinction of male and female eggs may be a general phenomenon, though not usually associated with dimegaly.

4. In parthenogenesis sex is necessarily determined without fertilization; from such eggs of Rotatoria, aphids, Phylloxerans and daphnids both males and females develop. Since there is no fertilization the daughter individuals should have the same chromosome complex as the parent, should all be females, were sex determined by particular chromosomes. Else there should be anticipated separations of particular chromosomes in definite manners, which would seem to imply most complex mechanical movements; as yet we know nothing definite of such movements.

5. In hermaphroditic species an egg gives rise to a bisexual individual, never to a unisexual. Were there sex determination by particular combinations of allosomes in the fertilized egg, we would necessarily expect occasional unisexual individuals to result. In *Sagitta* Stevens (1905) found an allosome in the spermatogenesis, but neither in oögenesis nor in the first cleavage; and she, as Cardiff (1906) who described one in the plant *Salomonia*, points out that such an element can have no sexual value in these hermaphrodite species.[1]

6. As Wilson and others have realized, to regard particular allosomes as direct sex-determinants logically necessitates selective fertilization. Until a case of selective fertilization has been demonstrated, however, the discussion on this point had better be tabled.

7. Morgan has urged that it may be the mass rather than the quality of the chromosome substance that may be sex-determinative, provided that such substance is determinative at all. It is the general rule in insects that the male has less chromosome substance than the female, in having a single monosome, or a small and large idiochromosome in the place of two large ones. It might then be argued that such allosomes, by the difference in mass which they occasion, establish the prospective sex value.

[1]It is not actually proven that these bodies are of chromosomal nature.

This agrees with the fact that eggs which have given off both polar bodies and are not fertilized give rise to males, as in the Rotatoria (Whitney) and some Hymenoptera.[1] However, this does not necessarily imply that particular chromosomes are sex-determinative even quantitatively, but that the mass of all chromosomes collectively may be determinative.

8. The hypothesis neglects the part that other substances, such as the cytoplasm and the mitochondria, may have in sex determination.

9. The strongest objection to the hypothesis of particular chromosomes being specially sex-determinative remains to be discussed, and it may equally well be made against certain current explanations of heredity in general. There can be little question, at least in the present state of our understanding, that chromosomes are of great importance in cellular metabolism, and even evidence that they are in part enzyme masses. But these chromosomes, while preserving their continuity from generation to generation, which I hold to be abundantly established, are in no sense independent units, but parts of a larger whole, the "nuclear element," composed of the sum of the chromatin and linin. Further, this nuclear element is not an independent unit, but only a part, even if it be the most important part, of the cell whole. Thus the idea is erroneous to speak of the chromosomes as automatic units, for they are but parts of the cell or cell complex. The whole, as Whitman (1893) argued, cannot be the single cells or parts of them, but the entire inclusive organization. For the organism acts as a whole, not simply as the sum of many parts; it is the interrelation of the activities of the many parts, added to these, that constitutes the behavior of this major unit.

Now to assume that particular chromosomes alone are sex-determinants is to disregard this complex inter-activity. At the

[1] It is now fairly well established that drones of the honey-bee, hornet, wasp and ant all possess the reduced number of chromosomes, and therefore must have originated from unfertilized eggs that had produced two polar bodies. The work of Meves (1907, 1908), Mark and Copeland (1906, 1907), Lams (1908) and Schleip (1908) is thoroughly corroborative of this conclusion. But this does not prove that in the Hymenoptera all unfertilized eggs give rise to males, for there seem to be certain established records of females resulting from unfertilized eggs, which cases have been collected from the literature by Wheeler (1903) and Shull (1910).

most we are justified in concluding only that the chromosomes have a share in the establishment of sex. He would be rash who would venture to claim that a particular chromosome determines excretion, another determines locomotion; yet these processes are relatively simple compared with that of sexuality, which some have contended may be controlled by a particular chromosome. The hypothesis is too naïve, it assumes too great simplicity of the cell, it tastes too strong of rigid predetermination.

The idea of unit characters, promulgated mainly by the work of Mendel, DeVries and their followers, is largely to blame for such hypotheses. It seems to me that physiological study has sufficiently demonstrated that there are no actual unit characters, and it is but natural that physiologists have refused to accept them. In the analysis of cross-breeding, the investigator has to focus his attention upon one or but a few characteristics of the organism; he has to close his eyes to the great multitude of characteristics, for they are too numerous for any one mind to grasp at once. The characters he may select for examination are his units of study, and he is entirely justified in speaking of them as unit characters, provided he does not forget that they are merely arbitrary units of convenience. But most hybridists have gone further than this; they have sought to directly compare such arbitrary excerpts with units of organization, scarcely pausing to consider what is a unit of organization. Surely it is the organism as a whole that is the only unit, and just as surely all its parts are most complexly interrelated. The living body is a unity, not a colony.

Modern Mendelian explanations represent a determinant theory far more rigid and complex than that of Weismann, though, strangely enough, most Mendelists in the inception of their studies were unsympathetic to Weismannian interpretation. This is the most curious instance of how men have come to identify an arbitrary term of convenience with a part of the living organization.

When Sutton (1903) pointed out that the paternal and maternal chromosome series parallel in their pairing and separation phenomena of alternative inheritance, thus seeming to present a cellular basis for Mendelism, and Castle (1903) argued that sex

follows such inheritance, the thought originated of identifying unit characters with chromosomes. It was made to appear that unit characters are present in the germ, though just what relation a rose comb has to a particular chromosome was not elucidated. Such a concatenation of ideas as this naturally led to the identification of "sex-units" with certain chromosomes.

The better founded idea that the organism behaves as a whole, whether it be a germ cell or a multicellular body, should make us hesitate to localize any particular function solely in one particular structure, for that would mean to disregard the importance of interrelations of parts. Thus when we find particular chromosomes in one sex and not in the other, it by no means follows that these are the cause of the sex difference. All we can say at the present time is that the two phenomena are coincident. Thus I am inclined to agree with Morgan's (1909) closing thoughts: "The accessory (chromosome) may follow sex or be associated with other differences that determine sex, rather than be its sole cause."

In all probability the activities of the chromosomes are influential in establishing sex, but not in the crude way in which the process has been imagined.

One point is quite clear, that fertilization is not necessary for the establishment of sex, for any unfertilized egg that develops furnishes a sexual individual. At the same time sex may be changed by fertilization; thus Whitney (1909) has shown it to be probable that the male eggs of Rotatoria furnish males if not fertilized, but females when fecundated. Sex is then established before, but may be changed by fertilization. This clearly implies that maleness and femaleness are not unchangeable unit characters, as does also the fact that an individual of one sex may develop some of the characteristics of the other sex, a phenomenon so apparent in the human body. Maleness and femaleness would appear to be two modes of one process, the process of germ cell production, not radically different conditions. In other words, there is no valid reason to interpret sex as an immutable unit character resident in or presided over by particular chromosomes, and sorted out and distributed by Mendelian segregation with all the complex mechanisms of dominance and

determiners; but rather as a growth, the result of a labile process which may be changed by a variety of influences.

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EXPERIMENTS WITH CHRYSOMELID BEETLES.[1]

ROBERT W. HEGNER.

Results obtained by the writer within the last five years from experiments upon *Calligrapha bigsbyana* and other chrysomelid beetles have shown that the eggs, larvæ, pupæ and adults of these insects are admirably suited for the study of many of the external and internal factors of development. The eggs may be definitely oriented with regard to the future position of the embryo quite easily; they continue to develop when subjected to extremely violent mechanical conditions; and various parts may be removed without retarding their development (Hegner, 1908*a*, 1908*b*, 1909*a*, 1909*b*). The larvæ are easily reared in the laboratory in an environment similar to that which they encounter in nature; they usually thrive well under experimental conditions. The same things may be said of the pupæ and adults.

It is the writer's intention to present in this paper, and those that are to follow, the data and conclusions derived from experiments dealing with the growth and external and internal factors which influence the development of the various stages in the life history of certain chrysomelid beetles. Thus far the willow beetles, *Calligrapha bigsbyana* and *C. multipunctata*, have received the largest share of attention, but other species have also been employed.

I. The Normal Rate of Growth of *Calligrapha Bigsbyana*.

1. *The Weight of Developing Eggs.*

Method and Data.—Two series of weighings were made to determine the loss of weight of developing eggs of *C. bigsbyana*. Table I. shows the results for twelve eggs laid by four different beetles at practically the same time. Two batches of two eggs each, one of three, and one of five were taken at 1 P.M. on June 29, placed in a watch glass, and covered by another watch glass. The loss in weight is quite striking. The pigmentation of the

[1]Contributions from the Zoölogical Laboratory of the University of Michigan, No. 128.

embryos became visible through the chorion on the sixth day (July 5). However, the eggs did not hatch, as is usually the case, on the fifth or sixth day (Hegner, 1908*a*), nor on the seventh or eighth day. Loss of water no doubt prevented the larvæ from breaking through the chorion.

TABLE I.

WEIGHINGS MADE OF 12 EGGS OF *Calligrapha bigsbyana* LAID BY 4 BEETLES AT 1 P.M., ON JUNE 29, 1909. AVERAGE TEMPERATURE, 23°C.

Date.	Total Weight in mgs.	Average Weight per Egg in mgs.	Total Loss in mgs.	Average Loss per Egg in mgs.
June 29	9	.75		
June 30	8	.6666	1	.0834
July 1	7.2	.6	.8	.0666
July 2	6.7	.5583	.5	.0417
July 3	6.1	.5083	.6	.05
July 4	5.8	.4833	.3	.025
July 5	5.1	.425	.7	.0583
July 6	4.4	.3666	.7	.0583
July 7	3.8	.3166	.6	.05

TABLE II.

WEIGHINGS MADE OF 22 EGGS OF *Calligrapha bigsbyana* LAID BY 5 BEETLES AT 4 P.M. ON JULY 7, 1909. AVERAGE TEMPERATURE, 25° C.

Date.	Total Weight in mgs.	Average Weight per Egg in mgs.	Total Loss in mgs.	Average Loss per Egg in mgs.
July 7	15.2	.6909		
July 8	15	.6818	.2	.0091
July 9	14.8	.6727	.2	.0091
July 10	14.1	.6409	.7[1]	.0318
July 11[2]	13.5	.6136	.6	.0273

It has been my custom to keep a small piece of filter paper, moistened with distilled water, in the watch glass with the eggs to prevent desiccation. Too much moisture frequently enables a fungus to establish a growth upon the chorion, sometimes checking the hatching of the eggs, whereas too little moisture may also prevent hatching. In nature the eggs are laid on the under surface of leaves where they are kept sufficiently moist by the condensation of water vapor at night.

[1]The moistened filter paper was removed on July 9th and returned on July 10. This accounts partly for the comparatively great loss in weight during this interval.

[2]Twenty of the eggs hatched on July 12. The chorions from which the larvæ escaped weighed 1.176 mg., or .0588 mg. per chorion.

The eggs whose egg weights are recorded in the second series (Table II.) were placed in a covered watch glass along with a piece of filter paper which was moistened with a drop or two of water every day. The loss in weight of these twenty-two eggs during embryonic development was not nearly so great as was that of the first series (Table I.), and doubtless represents more closely the state of affairs under normal conditions. Twenty of the twenty-two eggs hatched on the fifth day, the usual time for eggs of this beetle.

Discussion and Conclusions.—The belief has been held for many years that eggs diminish in weight during the early embryonic stages, and before extraneous food is consumed. That this belief is well founded has been proved by careful experiments with the eggs of several species of animals.

Pott and Preyer (1882) have shown that the hen's egg loses weight during incubation. The amount of oxygen absorbed by the eggs equaled the amount of CO_2 excreted. This excretion, produced in the physiological processes taking place during incubation, does not, at least in this case, account for the loss in weight, as is usually supposed, since the decrease is equalized by the absorption of oxygen. The conclusion was reached that a gradual evaporation of the albumen caused the loss in weight.

When hens' eggs are incubated in desiccators the rate of development is accelerated during the first three days, but later is retarded, and many of the embryos become abnormal or die (Féré, 1894).

Eggs that develop in water have also been used to determine the loss in weight of developing eggs during development (Ritter and Bailey, 1908). Bailey used for his experiments the eggs of the California mud-fish, *Fundulus parvipinnis*. Starting ten days after ensemination, 93 eggs were weighed at intervals of about 20 hours, covering a period of 9 days. Of the 36 weighings made, only 10 showed a gain, and this was accounted for by the presence of dirt upon the eggs. Bailey believes that the "loss in weight must have been due to carbon dioxide (CO_2) and organic salts representing the albuminoid loss, which had passed out through the egg-membrane and been washed away in the sea-water."

A loss of energy also takes place during segmentation, and, in

the case of the sea-urchin egg, has actually been measured, though not enough experiments were performed to make the resultant figures of much value (Spaulding, 1907).

The eggs of chrysomelid beetles differ in several respects from any thus far used for weight experiments. In the first place they are covered by a chitinous chorion which is comparatively impervious to fluids, and is especially well adapted to withstand desiccation. The method of cleavage, *i. e.*, superficial, differs from that of the eggs heretofore examined.

The results of the two series of weighings recorded in Tables I. and II. prove conclusively that there is a loss in weight, and that this loss is largely due to evaporation. A comparison of the data in Tables I. and II. shows this quite clearly, since the eggs weighed in Table I. were allowed to develop without the addition of moisture, and consequently decreased in weight more rapidly.

2. *The Rate of Growth of Larvæ, Pupæ and Adults.*

Method and Data.—The twenty larvæ that hatched from the eggs used in determining the loss in weight of developing eggs (see Table II.) were weighed daily until they pupated; the pupæ were then weighed daily, and finally the adults. These weighings extended over the period from July 12 to August 14, 1908. Because of the daily disturbances made necessary by the weighings, many of the larvæ died. This mortality was greatest during the first four days; however, under normal conditions, many of the larvæ die during this early stage.

The data obtained have been arranged chronologically in Table III. Fig. 1 gives the curve showing the daily increase in weight and Fig. 2 gives the curve showing the daily percentage increments in weight.

Discussion and Conclusions.—The problem of growth is one of great interest to zoölogists, and its study has been given added impetus by the work of Minot (1891, 1907). This investigator considered growth not as in increase in size or volume, but as an increase in mass or weight. The rate of growth was measured by him by taking the increase in weight during a definite period and expressing it as a percentage of the weight at the beginning of that period. Any change in weight can thus be shown by successive percentages for equal periods of time.

TABLE III.

THE RATE OF GROWTH OF LARVÆ, PUPÆ AND ADULTS OF *Calligrapha bigsbyana*.

Date 1911.	Age in Days.	No. of Larvæ.	No. of Pupæ.	No. of Adults.	Total Weight in mg.	Average Weight in mg.	Daily Increase in Weight in mg.	Per Cent. Daily Increase.
July 12		20			11.3	.565		
July 13	1	20			12.	.6	.035	6.2
July 14[1]	2	20			12.6	.63	.03	5.
July 15[2]	3	17			19.	1.118	.488	77.4
July 16[3]	4	9			15.6	1.733	.615	55.
July 17	5	9			17	1.889	.156	9.
July 18	6	9			34.2	3.8	1.911	101.1
July 19[4]	7	9			39.4	4.377	.577	15.2
July 20[5]	8	8			44.6	5.575	1.198	27.3
July 21	9	8			58	7.25	1.575	28.2
July 22	10	8			107	12.375	5.125	70.7
July 23[6]	11	8			134	16.75	4.375	35.3
July 24	12	8			140.5	17.582	.832	4.9
July 25	13	8			193.5	24.187	6.605	37.5
July 26	14	8			287	35.875	11.688	48.3
July 27[7]	15	7			294.8	42.114	6.239	17.4
July 28[8]	16	7			284	40.57	— 1.544	— 3.6
July 29[9]	17	6			249	41.5	.93	2.2
July 30	18	6			235	38.917	— 2.583	— 6.2
July 31	19	6			257	42.833	3.916	10.
August 1	20	6			257	42.833	0	0
August 2	21	6			259	43.166	.333	.77
August 3	22	6			250	41.666	— 1.5	— 3.4
August 4	23	6			236	39.33	— 2.333	— 5.6
August 5	24	6			239	39.833	.5	1.2
August 6	25	4	2		242	40.33	.5	1.2
August 7	26	3	3		238	39.66	— .67	— 1.6
August 8	27	3	3		235	39.166	— .5	— 1.2
August 9	28	2	4		234	39.	— .166	— .42
August 10	29	2	4		235	39.166	.166	.42
August 11	30	2	4		234	39.	— .166	— .42
August 12	31	1	2	3	231	38.5	— .5	— 1.25
August 13[10]	32		2	3	194	38.8	.3	.77
August 14	33		2	3	190	38.	— .8	— 2.06

[1]Larvæ began to feed.

[2]Three larvæ died.

[3]Moulting began on the fourth day; eight larvæ died.

[4]Second moult in progress.

[5]One larva died.

[6]Third moult in progress.

[7]One larva died.

[8]Feeding practically stopped and larvæ prepared for pupation.

[9]One larva died.

[10]One larva died.

Minot's results from weighings made of guinea-pigs show that the growth rate increases almost immediately after birth, the decline being very rapid at first, but less rapid as the age of the

animals increases. That there is a corresponding prenatal decline in the rate of growth was shown by means of rabbit embryos. Curves representing the change in the rate of growth with age

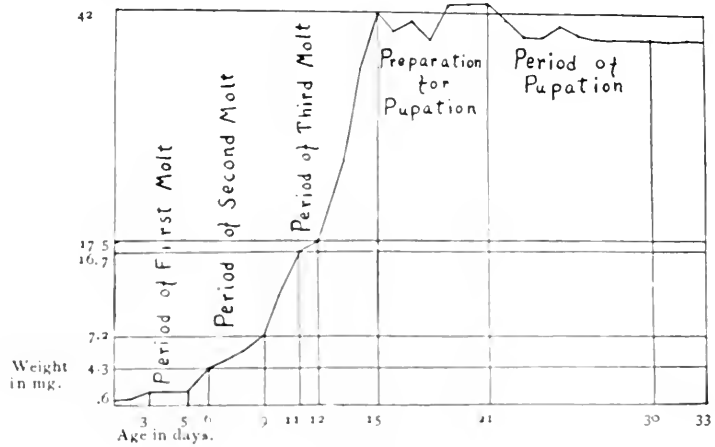

FIG. 1.

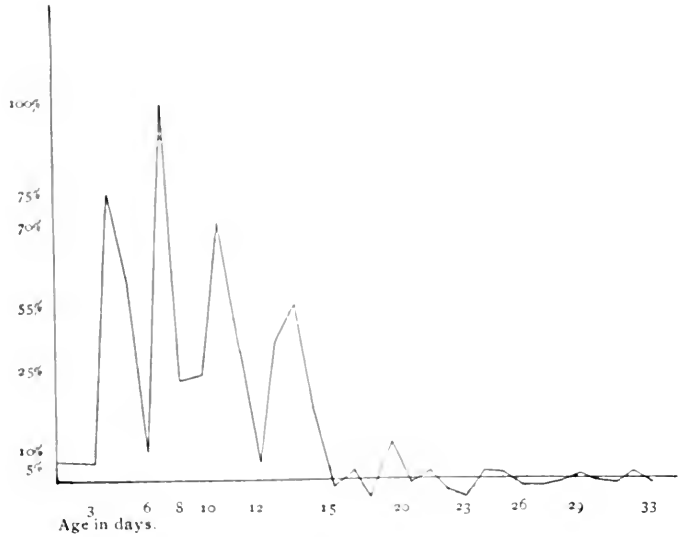

FIG. 2.

have been constructed for the embryos and young of many animals, and almost without exception the growth-rate declines as development proceeds.

Davenport (1897) has shown for the tadpoles of *Rana*, *Bufo* and *Amblystoma*, that, during the first two weeks of larval life, growth is largely due to the absorption of water, which increased from 56 to 96 per cent. During later development, however, the storing up of formed substances is mainly accountable for their growth. The curve of the growth-rate for tadpoles does not agree with the general rule; it rises first, then declines, and finally rises again. This result is probably due to the absorption of water.

TABLE IV.

THE RATE OF GROWTH OF LARVÆ OF *Telea polyphemus* (TROUVELOT, 1867).

Age in Days.	Weight in Grains.	Increase in Weight in Grains.	Per Cent. Increase.
Just hatched	.05		
10	.5	.45	900
20	3	2.5	500
30	31	28	933
40	90	59	190
56	207	117	130

Trouvelot (1867) has given a few weighings of the larvæ of the moth, *Telea polyphemus*. These have been arranged in Table IV. so as to show the actual increase in weight, and also the percentage increments for ten day intervals. The decline in the rate of growth is not regular, probably because of the meager data, but it is no doubt similar to that exhibited by the guinea-pig and other animals.

Fig. 1 shows the weight of developing beetles of the species *C. bigsbyana* from the time of hatching to the emergence of the adults, a period of 33 days. The following data will make clear certain irregularities in the curve. The larvæ usually devour a part or all of their cast-off egg-shells soon after hatching, but do not begin to feed actively until the second day; this accounts for the very slight increase in weight during the first two days. An actual decrease in weight would be expected at the moulting periods, when food-taking ceases and the chitinous covering is shed, but all larvæ do not moult at the same time (see Table V.), and instead of a decrease in the average weight, there is a slight increase. This is shown in all of the moults. The period of most rapid increase is that between the fifth and the fifteenth

TABLE V.

THE WEIGHT OF INDIVIDUAL LARVÆ OF *Calligrapha bigsbyana* WHEN 7 DAYS OLD; JUST AFTER THE FIRST MOULT.

Date of Moulting.	Weight in mgs.
July 16	5.8
July 16	5.4
July 16	5.4
July 17	4.8
July 17	4.6
July 17	4.
July 18	2.6
July 18	2.
July 19	.9

days. From the latter time onward the larvæ gradually cease feeding and lie on their backs in the earth provided for them. During this preparation for pupation, and during the period of pupation, there is a steady decline in weight until the adults emerge.

Fig. 2 shows the daily percentage increments in the weight of the developing beetles. The remarks made in explanation of Fig. 1 also explain the irregularities in this curve. The percentage increments decline very rapidly during the moulting periods. If all of the larvæ moulted at the same time, the rate would be negative.

The data obtained from these weighings confirm what Minot (1891, 1907) has found to be true of guinea-pigs, *i. e.*, the rate of growth declines rapidly during the early stages of development and more slowly during the later stages. Jenkinson (1909) has obtained similar results for many other animals by using the data already available in literature.

II. THE EFFECTS OF LIGHT UPON THE DEVELOPMENT OF *Calligrapha Bigsbyana*.

1. *The Influence of Darkness.*

Method and Data.—Experiment C.B. 42. Eight eggs of *C. bigsbyana* were laid at 12 M., June 10. Four were allowed to develop in an ordinary stender dish (7 cm. in diameter), and the other four were placed in a similar receptacle which had been covered externally with a coat of opaque paint. The same amount of moisture was supplied to each dish, and the temperature did not vary a degree.

Two eggs in the light and all of those in darkness hatched on June 19; the two remaining in the light hatched on June 20. On June 23 two of the larvæ in the darkness moulted. Three of those in the light died on June 23; the other moulted on June 24, as did the two remaining in the dark. All of the larvæ were accidentally destroyed on June 25.

Experiment C.B. 70. Four batches of eggs were laid by four different beetles at approximtaely 10:30 A.M. June 26. One half of each batch were allowed to develop in the light several feet from a window; the other half were placed in darkness as in experiment C.B. 42. The conditions of moisture and temperature were similar in the two dishes. The data have been arranged in Table VI.

TABLE VI.

DATA RECORDED IN EXPERIMENT C.B 70, SHOWING THE RATE OF DEVELOPMENT OF EGGS, LARVÆ AND PUPÆ OF *Calligrapha bigsbyana* IN WHITE LIGHT AND IN DARKNESS.

Date 1909.	White Light.	Darkness.
June 26	15 fresh eggs from 4 batches	16 fresh eggs from 4 batches
July 1—8 A.M.	5 hatched	5 hatched
July 1—1 P.M.	2 hatched	2 hatched
July 2	7 larvæ	2 hatched
July 3	7 larvæ	9 larvæ
July 4	7 larvæ	8 larvæ alive 1 larva dead
July 5	1 moulted	
July 6	4 moulted	6 moulted
July 7	1 second moult 1 still in 1st instar	1 dead
July 8	2 second moult 1 still in 1st instar	1 second moult 1 dead
July 18	5 ready to pupate	4 ready to pupate
July 21	2 pupæ	1 pupa
July 22	4 pupæ	4 pupæ
July 23	5 pupæ	5 pupæ
July 26	5 pupæ	6 pupæ
July 28	2 adults	1 adult
July 29	3 adults	3 adults
July 30	5 adults	5 adults
August 1	5 adults 2 larvæ did not pupate	5 adults 1 adult did not emerge

Discussion and Conclusions.—The eggs of *C. bigsbyana* are attached to the under surface of the leaves of the food plant of the larvæ, *Salix longifolia*, and are thus never exposed to the direct rays of the sun except for exceedingly brief intervals when the leaves twist in the wind. They develop therefore in light of

moderate intensity. Eggs that develop within an opaque mother, or that possess an opaque envelope, pass through their embryonic stages in darkness; but there can be no doubt that the chorion of the beetle's egg allows the light to penetrate, since, as I shall show in a later paper, sunlight has a decided influence upon embryonic development.

In certain cases experiments have seemed to prove that darkness delays the growth of the eggs or larvæ, *e. g.*, Yung (1878) recorded not only a retardation in the development of frog larvæ, but also a high death rate. The same investigator noted a slight retardation in the development of the eggs of the snail, *Lymnæa stagnalis*, when placed in the dark.

Vernon (1895), on the other hand, found that echinoderm larvæ suffer very little, if any, change from the normal when reared in absolute darkness. Loeb (1896) also has brought forth evidence proving that darkness does not retard the embryonic development of the fish *Fundulus*, but does effect a decrease in the number of pigment cells on the yolk-sac.

In other cases, darkness does not hinder the growth of the embryo or larva, but fails to stimulate the hatching process. Przibram (1906) found that the larvæ of the praying mantis, *Sphodromantis bioculata*, are retarded if the cocoon is placed in the dark.

In discussing experiments C.B. 42 and C.B. 70, the normal rate of development and its variations must be noted. Records of over 2,000 eggs of *C. bigsbyana* and the closely allied species *C. multipunctata* give 5 days and 16 hours as the average hatching period (Hegner, 1908*a*). This period varies according to conditions of moisture, temperature and probably other external factors, from 4 to 7 days. Records were also made of over 1,000 larvæ. The average larval life is 20 days; but, as in the case of the hatching time, this period may be shortened to 17 days or extended over 24 days by differences in external conditions. The average pupal period is 12 days, though adults frequently emerge in a shorter time, and a few do not escape until 13 or 14 days have elapsed. These variations in the duration of the different stages may occur in eggs, larvæ or pupæ from different batches of eggs or from the same batch.

The data from experiments C.B. 42 and C.B. 70 indicate that darkness has no retarding nor accelerating influence upon the embryonic development, upon the rate of larval growth, or upon the period of pupation.

One other conclusion that may be arrived at from these experiments is that darkness has no effect upon the coloration of the eggs, larvæ, pupæ or adults of the species studied. Frequent examinations were made during the growth of the beetles reared in the dark, but no variations from the normal were discovered that could be attributed to the absence of light. This confirms Przibram's (1906) results for the praying mantis, the entire post-embryonic development of which was carried out in the dark without producing any effect upon the coloring.

2. *The Influence of Colored Lights.*

Method and Data.—Experiment C.B. 64. This experiment is the only one attempted with a view to testing the effects of colored lights upon the embryonic development of beetles' eggs; but it indicates that color has no very striking influence upon the rate of development.

Several eggs from a single batch of 15, which were laid at 10:30 A.M. on June 24, were placed in each of six cylindrical tubes. These tubes were then closed with rubber corks through each of which were inserted a thermometer and a tube for ventilation. These cylindrical tubes were then immersed in different colored liquids prepared according to Yung (1878). The colors used were red, blue, yellow, green and violet, and a tube was kept in pure water as a control. The temperature in the different tubes was practically identical. The eggs in the white, yellow, green and red lights hatched on June 29; those in the violet and blue were ready to hatch on the same day, but were prevented by fungus growths.

Discussion and Conclusions.—Many experiments have been performed with eggs of a number of species of animals to determine the influence of colored lights upon their development. Yung (1878) used freshly laid eggs of the frog, *Rana esculenta* and *R. temporaria*. At the end of two months all of the tadpoles in the green light were dead, those in the white and yellow lights

were greater in number, those in the red light were retarded and finally died, and those in the violet light were larger, but less advanced and had greater powers of resistance.

These results have not been confirmed for the frog and other animals by later investigators. For example, Vernon (1895) found that the larvæ of echinoderms, in some cases, were not killed by the green light, and that yellow light caused greater injury than red. Driesch (1892), moreover, claims that the eggs of *Rana*, *Echinus* and *Planorbis* are not influenced by any of these colors.

In experiments on the praying mantis, Przibram (1906) found that the influence of green, red and yellow glasses was unfavorable, though this may have been due to differences in the temperature, which was not controlled.

My experiment with the eggs of *C. bigsbyana* confirms for the eggs of this beetle the results obtained by Driesch for eggs of *Rana*, *Echinus* and *Planorbis*.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF MICHIGAN,
April 15, 1910.

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THE MARSUPIUM OF THE UNIONIDÆ.

GEORGE LEFEVRE AND WINTERTON C. CURTIS.

In a recent preliminary announcement of a new system of classification of the Unionidæ which is based upon a study of the anatomy of the fresh-water mussels of Pennsylvania, Ortmann[1] suggests a division of the family (exclusive of the Hyriinæ) into four subfamilies, namely, Margaritaninæ, Unioninæ, Anodontinæ and Lampsilinæ, respectively. His arrangement lays especial emphasis on the characters of the marsupium and involves several important modifications of Simpson's classification, which he maintains must be recast to a considerable extent in order to make it represent the natural affinities of the group. Although it will not be possible to form an opinion in regard to the validity of the proposed changes before the appearance of his detailed results, he has undoubtedly fallen into a serious error with respect to the marsupium of one of his subfamilies, and it is the object of the present note to point out this mistake.

In connection with the fresh-water mussel investigations which have been under way in this laboratory for some time and which have been carried on for the United States Bureau of Fisheries, primarily for the purpose of determining the feasibility of artificial propagation of the Unionidæ, we have had occasion to give attention to the anatomical and histological structure of the marsupium in a large number of genera, and, furthermore, we have been particularly concerned with the changes that occur in the gills during the period of gravidity. Since a fundamental discrepancy exists between Ortmann's description of the gravid gill in his subfamily Anodontinæ and our own observations on at least three of the genera which he includes in this group, namely, Alasmidonta, Anodonta and Symphynota, we have thought it advisable to call attention to the fact.

Ortmann (p. 117) makes the following rather astonishing statement concerning the structure of the marsupium of the Anodon-

[1] "A New System of the Unionidæ," A. E. Ortmann, *Nautilus*, XXIII, February, 1910, pp. 114–120.

tinae: "Water-tubes in the gravid female *divided longitudinally into three tubes*, one lying toward each face of the gill, the third in the middle; only the latter contains eggs or embryos, and is much larger than the other tubes. This division into three parts is not present in the sterile (*sic*) female." Although it is not specifically stated, it is to be inferred from the above description that the divisions of the water-tubes into three parts is due to

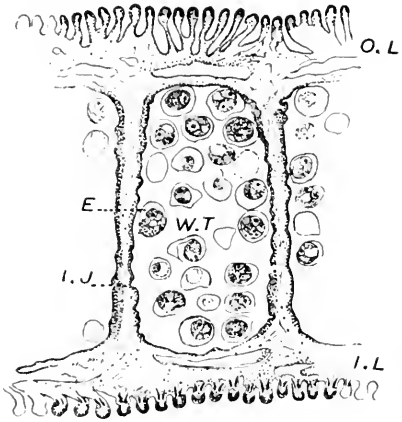

FIG. 1. *Anodonta cataracta* Say.—Horizontal section of portion of gravid marsupium, showing a water-tube, undivided and filled with embryos. *O.L*, outer lamella of gill; *I.L*, inner lamella; *I.J*, interlamellar junction; *W.T*, water tube; *E*, embryos, x31.5 Kline del.

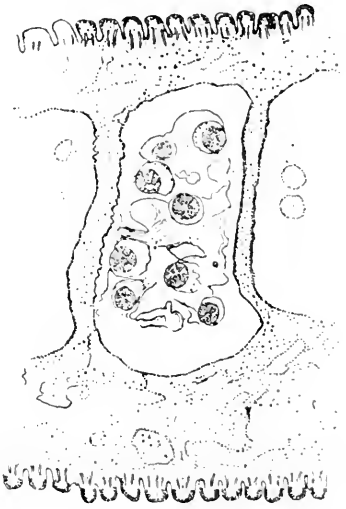

FIG. 2. *Alasmidonta truncata* Wright.—Horizontal section of portion of gravid marsupium, showing a water-tube, undivided and filled with embryos. The mass of embryos is somewhat contracted into the middle of the tube. x 31.5 Kline del.

the presence of longitudinal partitions running parallel with the lamellæ, but no intimation is given as to how they arise, or how they disappear after the marsupium has discharged its contents. To any one familiar with the structure of the gills of the Unionidæ the statement that the water-tubes exhibit a temporary division into three parts is on its face improbable, for it would be difficult to imagine how such a division could be brought about, and still more difficult to understand why, when once established, it

should disappear after spawning. It is true that one occasionally encounters a partial fusion of two adjacent interlamellar junctions, with a consequent division into two or more parts of the water-tube lying between them, but this is not constant in occurrence for the species and, when it is present at all, it involves only a single tube here and there in the gill. We have observed such fusions in a few individuals belonging to different genera in both gravid and non-gravid gills, but it is a condition that must be regarded merely as an occasional variation and is entirely different from that which is supposed by Ortmann to exist in the Anodontinæ. His description, moreover, is at total variance with our observations in the three genera referred to, as sections of the gills in these forms, taken at various stages during the gravid period, show *absolutely no trace of such a division of the tubes*. In Figs. 1–3, which are drawn from horizontal sections of the gravid marsupium of *Anodonta cataracta* Say, *Alasmidonta truncata* Wright and *Symphynota complanata* Barnes, respectively, the water-tubes, containing embryos and glochidia, are seen in their usual form, undivided longitudinally and bounded by the inner and outer lamellæ of the gill and by he interlamellar junctions. If such a division of the tubes into three parts, as Ortmann describes, were present, it would of course be indicated in these sections.

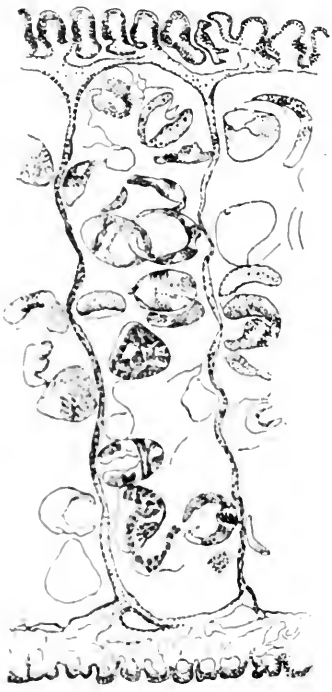

FIG. 3. *Symphynota complanata* Barnes.—Horizontal section of portion of gravid marsupium, showing a water-tube, undivided and filled with glochidia. x 31.5 Kline del.

We are at a loss to understand what appearances observed by Ortmann could have given rise to his error. The only thing that suggests itself is that the material which he used had been

badly preserved and the gills in consequence much shrunken. In this event, it is quite possible that the embryos might have been contracted into a mass in the middle of the water-tube and the mucus, by which they are surrounded, coagulated in such a way as to cause the appearance of septa stretching between the interlamellar junctions when observed under a low magnification. It is not uncommon to find the embryos contracted in this manner to a greater or less degree as a result of fixation, as may be seen in Fig. 2, in which the mass of embryos has been withdrawn slightly from the inner surface of the lamellæ. The fact that he states that the divisions are only present in the gravid gills would lend some degree of plausibility to this explanation.

Zoölogical Laboratory,
University of Missouri,
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THE "PYLORIC GLAND" OF THE ASCIDIAN BOTRYLLUS—AN ORGAN OF EXCRETION?

HAROLD SELLERS COLTON,

UNIVERSITY OF PENNSYLVANIA.

INTRODUCTION.

This contribution deals with the anatomy and physiology of an organ found in most of the groups of the Tunicata. This organ is usually composed of a system of fine tubes which ramifying over the walls of the intestine, are joined to some portion of the stomach by one or more ducts. Although of general occurrence in the Ascidiacea and Thaliacea, yet there has been no concurrence of opinion as to its function. In consequence of this fact hardly two authors have referred to it by the same name. Hence we have this organ figured as (1) glandes diverses (Savigny, 1810)[1] and referred to, if we leave out literal translations as, (2) liver (Hancock, 1866) (Krohn, 1852) (Milne-Edwards, 1841),[2] (3) glande annexe du tube digestif (Chandelon, 1875) (v. Winiwarter, 1896), (4) glandola epato-pancreatico (Della Valle, 1881), (5) lacteal system (Lister, 1834) (Huxley, 1851) (Ritter, 1896), (6) intestinal gland (Herdman, 1882) (Maurice, 1888), (7) lacunes stomaco-intestinales (Roule, 1884), (8) glande stomacale (van Beneden et Julin, 1884), (9) darmumspinnende Drüse (Seeliger, 1882) (Dahlgrün, 1901) (Isert, 1903), (10) organe réfringent (Giard, 1872) (Pizon, 1893) and (11) glande pylorique (Lacaze-Duthiers et Delage, 1889) (Willey, 1893).

With such a choice of names what shall we call the organ in question? Since the name pyloric gland is short and sufficiently non-committal and has been dignified by usage, we will use it in preference to the others.

The following study is an outgrowth of one that the writer has been at work on for the past two years, and as it has developed into slightly other lines than was originally planned, he has taken this excuse to make a separate paper of this portion. The work

[1]See Chandelon, 1872.

[2]See Giard, 1872*a*.

as a whole was begun in the zoölogical laboratory of the University of Pennsylvania. It was continued at the Zoölogical Station at Naples, at the Fisheries Laboratories at Woods Hole, and Beaufort, and the following part of it completed in the Zoölogical Laboratory of the University of Pennsylvania. At this point the writer wishes to express his great thanks to the Carnegie Institution for the use of one of their tables at the Naples Laboratory, to the authorities of the station for their many kindnesses and hospitality, to the United States Commissioner of Fisheries for the use of a table for two weeks at Woods Hole, and one for two weeks at Beaufort, and also to the directors of those stations, Dr. F. B. Sumner and Mr. H. D. Aller in particular.

Although for this study most of the living material was procured in the salt-water tanks of the vivarium of the University where *Botryllus* colonies have been established for many years, yet the wealth of material preserved in Naples and Woods Hole has often been called into requisition while living material of other families of ascidians were studied at Beaufort.

According to Bancroft ('03) there is but a single species of *Botryllus* found in the north Atlantic Ocean and its extensions. Many have been described, but they are found to be based on color variations and habit of growth depending partly on the age and partly on the physiological state of the colony. The writer having worked at both Naples and at Woods Hole supports the view of Bancroft and considers that *Botryllus schlosseri* (Pallas) Savigny, is the form represented on both sides of the ocean.

The material was fixed in Flemming's solution, in corrosive sublimate, sublimate acetic, formol, etc. The best results were procured with Flemming's solution. Sections were cut 6 μ and stained in Delafield's hæmatoxylene and eosin. However, most of this study was made on the living animals and sections were used only to check up the results.

Morphology.

The alimentary tract of *Botryllus* is of the typical ascidian type and may be represented by the letter U of which one arm will be the œsophagus and stomach while the other is represented

by the intestine and rectum (Fig. 1). In the angle between the two arms lies a small blind sac, an out-pocket from the pyloric end of the stomach. Its walls are thick and glandular, similar to the walls of the stomach, but the cells that compose it do not contain the secretion that gives the stomach its characteristic yellow color.

This organ was called by Lahille ('90) the pyloric cœcum. It is into this sac, at the point where it enters the stomach, that the duct of the pyloric gland empties.

If we follow the duct from the point where it enters the pyloric cœcum and trace it to the intestine we will find it divide just before reaching that organ, sending a branch both to the right side and to the left side. At once on reaching the walls of the intestine both branch many times, finally ending in blind bulbs or ampullæ. However, all the branches do not end thus, but a few—not more than five or six—proceed half way to the anus, often without branching again. These tubes do not end in ampullæ. As they reach the region of the rectum in some cases, we will for convenience refer to them as rectal tubules.

It is very easy to study the organ in the living *Botryllus* and indeed it is possible in that way to see much more than can be observed in the preserved material, either in sections or in surface view. When a cormus is removed from its substratum and placed on a microscopic slide, the stomach and intestine can be teased out with a pair of needles under a dissecting microscope. The cormus is then removed from the slide, a drop of sea water added and the whole covered with a cover glass. The writer found artificial light in the shape of a Welsbach burner, a Zeiss apochromatic 2 mm. objective, and compensating oculars 8 × and 12 ×, necessities in the present study.

In the living tissue the tubes and bulbs appear highly refractive. In some cases it is quite easy to see the nuclei and even the chromatin in the nuclei. In many cases cell boundaries are quite clear and the presence of cell granules is easy to determine.

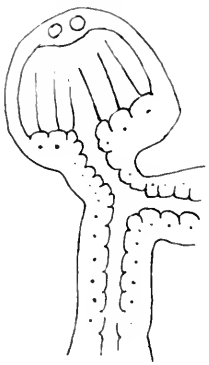
The ampullæ are bounded by a rather flat epithelium, the cell walls of which contain often refractive granules of a yellow color. The cells bear long whip-like flagella, but it is difficult to determine if all are so provided. These flagella soon lose their move-

ment after being placed on a slide, although the writer has observed them beating for five hours after the alimentary tract was removed from the organism. Fig. 2 represents the character of the epithelium and flagella in two adjoining ampullæ.

In *Botryllus* this organ was reported ciliated by Della Valle ('81) who wrote, p. 458: "La struttura intima di questa glandola è simplicissima trattadosi d'un semplice epitelio, che io ho veduto sempre sfornito di cigli vibratili." He gives no figure.

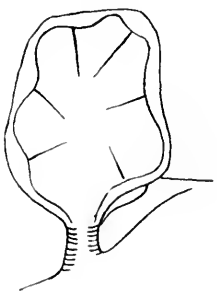
Pizon ('93) particularly mentions that he is unable to verify Della Valle and finds the lumen of the ampullæ unciliated. There are three other cases in which the pyloric gland has been found ciliated—Chandelon ('75) in *Perophora*, text Fig. 1, Uljanin ('84) in *Doliolum*. text Fig. 2 and Isert ('03) in *Microcosmus*. The

FIG. 1.



After Chandelon.

FIG. 2.



After Uljanin.

Ampullæ of the pyloric gland as seen in *Doliolum* and *Perophora* respectively.

latter found the ducts and believed the ampullæ were ciliated too, although he could not see it. The cases in which cilia have been seen in the ampullæ were all observed before the modern methods of microscopic technique were evolved. Since then we have been carried away by the use of dead material when in many cases, perhaps, as much if not more could be seen in the living.

Although the protoplasm of the walls of the ducts and ampullæ seems to be clear and refractive in the living animal, yet there are here and there yellowish granules in the cells. These are exceedingly minute, .1 to .2 μ in diameter, and are found in that portion of

the cell nearest to the lumen of the gland. One rarely sees more than a single granule in a cell. Among these small granules are larger ones, .5 to .8 μ, distinguished from them by being more refractive. In character they seem much like the brown concretions found in certain blood cells (Fig. 18). However, these concretions cannot be found in the preserved material, so the supposition is that they are composed of a material that is not coagulated by the killing fluid, this material being of the nature of a secretion. The concretions in the blood cells are not easily dissolved by any ordinary reagents used in microscopic technique therefore they are found in the preserved material.

At Beaufort the writer had the good fortune to be able to examine the ampullæ of the living *Amaroecium stellatum*, *Perophora veridis*, *Ascidia* sp., *Styela plicata* and *Molgula manhattensis*. All had flagella in the pyloric gland. In *Amaroecium* the flagella were similar to those found in *Botryllus*. In *Perophora* some ampullæ were like those found in *Botryllus* (Fig. 22), but others had exceedingly few cilia which were directed toward the mouth of the tube and not away as in Chandelon's figure (Fig. 21). Since the writer was able to observe movement in all the cilia in this ampulla their direction can be determined to a certainty. The walls of the ampullæ of *Ascidia* showed no essential character different from that of *Botryllus* (Fig. 19). In the cells of the gland of *Styela* (Fig. 24) is found the yellow secretion just as Isert described it in *Microcosmus* and in the lumen of the gland are found globules of it. In *Molgula* the organ is typical (Fig. 23). Since so many diverse families of ascidians show flagella in the lumen of the pyloric gland, the writer believes that if carefully looked for this organ in all tunicates will be found to bear flagella.

The walls of the tubules are similar to those of the ampullæ except that the cells are more cuboid (Fig. 3), and the flagella shorter. No granules are to be detected in the lumen of the tubes of *Botryllus* such as Isert found in the ducts of the pyloric gland of *Microcosmus*, and as the writer has found in the ducts of the gland in *Styela*.

When we compare the rectal tubules with the other portions of the organ in *Botryllus* several things may be noticed. Among

these things our attention is particularly called to the relative fewness of flagella and nuclei in the terminal portions of the tubules. Figs. 4–13 show flagella while Figs. 14 and 15 show nuclei. Although it is not possible to distinguish cell boundaries either in the preserved material or in the living tissue, yet the fewness of the nuclei might suggest the possibility that the cells composing this part of the gland have intracellular lumens. Again the terminal ends of these tubes exhibit two different types. We may have those unsegmented with a very small lumen, 2–6 μ (Figs. 8–12, 14 and 15), or we may have segmented tubes with a much larger lumen, 4–10 μ. In the first type of tube our attention is at once attracted to the terminal end of the tube. In most cases there seems to be a very thin place in the walls of the tubes. This thin place may be formed in three ways: (1) by the lumen of the tube approaching the exterior (Figs. 8, 14, 15), (2) by a cup-like depression in the end of the tube (Figs. 9-12, 15), (3) by a vacuole in the wall of the tube which does not communicate either with the exterior or the interior. To these three cases there is a fourth effect that the writer has observed. He thought that he could see tubes less than a micron in diameter that formed a direct communication between the interior of the tube and the blood space. The structures found at the end of the rectal tubules are so small that what we may interpret as a duct may be nothing more than a division between two cells—or a nucleus—both of which look clearer in preserved material and in the living tissue than the cytoplasm of the cell. The cup-like depression at the end of the tube suggests the organ of Boveri as found in *Amphioxus* or perhaps a nephridial funnel. The writer has searched the neighborhood about the ends of the tubes to see if he could find solenocytes as described by Goodrich ('09) in *Amphioxus* but without result. Again he has watched particles in the blood in the neighborhood of the possible opening, yet in no case has he been able to observe such a particle enter the tube. In this connection the experiment of Kupffer ('72) is interesting. He says (p. 381): "Mir ist es auch bei *Ascidia canina* gelungen, dieses System wenigstens partiell vom Herzen aus zu injiciren. Die Injectionsmasse war in mehrere der blinden Anhänge eingedrungen. Solche blinde kolbige Anhänge sind auch nichts Neues

im Gefässsystem der Ascidien. Man findet dasselbe an den colonialen Gefässen in der gemeinsamen Tunica der Synascidien. Ich halte daher das Ganze für einen besonders enwickelten Theil des Circulationsapparates dem wohl neben der Resorption des Chymus noch andere Functionen zukommen."

If *Ascidia canina* should have open communication between the lumen of these tubes and the blood cavity it would easily explain how Kupffer found the injection mass in the lumen of the gland. Since no communication has been demonstrated, it would be easier to explain the result of Kupffer in the light of the writer's own experiments with indigo carmin on *Styela* (p. 43). With the present evidence before us we cannot assume that there is any communication between the blood spaces of *Botryllus* and the lumen of the pyloric gland.

The cases in which cilia have been seen in the ampullæ were all observed before the modern methods of microscopic technique were evolved. Since then we have been carried away by the use of dead material when in many cases, perhaps, as much, if not more, could be seen in the living.

Pizon ('93) has studied the origin of this organ both in the tadpole and bud of *Botryllus*. Of this study the writer has verified the results and can but accept the conclusion of Pizon ('93) that in both cases the origin of the pyloric gland is from the endoderm by a simple diverticulum of the gut. This agrees perfectly with Van Beneden and Julin ('84 and '86) in *Phallusia*, Lefevre ('98) in *Perophora*, Ritter ('96) in *Goodsiria*, Seeliger ('82) in *Clavelina*, Uljanin ('84) in *Doliolum*, etc. In all cases it arises as an out-pocket of the stomach.

Experiments.

The smallness of the pyloric gland in *Botryllus* and the fineness of the tubes and ampullæ as found in the larger ascidians, together with the close application of the gland to the walls of the intestine, to the reproductive organs or to the renal vesicles, would forbid, in any form that has yet been available to the writer, direct physiological determinations. It is due to this that the nature of the organ has been problematical. To be sure Henri ('03) claims to have isolated the gland in *Salpa*, but as yet the

writer has been unable to procure the form in question. As no direct experiments on the nature of the fluid contained in the ducts and ampullæ have in this case been possible, the writer has resorted to indirect means—that of the use of certain in-vitam stains.

In the use of these stains the writer has not proceeded far and hopes at another time to undertake a fuller discussion of their significance. Suffice it to say that certain dyes, when introduced into the blood of a living organism in solution, have affinities for formed substances in the cells of certain tissues, as methylene blue in nervous tissue. Others, such as neutral red, act as indicators, telling us whether a given substance has an acid reaction or not, while still other dyes are segregated out of the blood as solids and deposited in cavities often connected with the exterior.

Following the experiments of Chrzonszezewsky ('64), Heidenhain ('74)[1] by injecting certain dyes, principally indigo carmin and ammonium carminate, into the veins of vertebrates came to the conclusion that the former dye was excreted by the Malpighian tubules of the kidney, while the glomeruli excreted the carminate. Kowalewsky ('89) carried this idea into his experiments on invertebrates, concluding that renal cells show either acid or alkaline reaction which determines the character of the secretion. However, Schmidt ('91)[2] has shown this idea false, as both ammonium carminate and indigo carmin may be excreted by the same organ. Nevertheless, it is a rather general characteristic of renal organs that they excrete carmin in some form.

In this study of *Botryllus* the writer has placed colonies in neutral red, in Bismarck brown, in ammonium carminate and in indigo carmin, studying the reaction of the pyloric gland to these dyes. Neutral red in concentrations rendering the sea water a pale yellow, stains the secretion in the cells of the organ an intense red and colors the liquid in the lumen of the ducts and ampullæ also. The probable significance of this is that the secretion has an acid reaction. Bismarck brown coloring the water much like that of the neutral red, stains the granules brown and

[1]Cited from Bruntz, '03.

[2]Cited from Bruntz, '03.

in contrast to the blood and to the sea water, the contents of the lumes of the tubes and bulbs is very brown. There are two alternatives by which to interpret these two experiments—either these stains act as indicators or they are actually concentrated in the lumen of the organ being separated from the blood by the cells of the walls of the tubes and ampullæ. Beyond certain leucocytes located in the neighborhood of the organ which seem to collect the dye, the only other cells that take up the ammonium carminate and indigo carmin are the vacuoles in the intestinal cells. A cross-section of the intestine has the shape of a rectangle (Fig. 17), the ends of which are made up of an epithelium of flat cells bearing cilia, while the sides are thick and are composed of two sorts of columnar cells, ciliated and glandular (Fig. 14). The gland cells under ordinary condition contain a clear liquid. It is the vacuoles of these gland cells which stain slightly in ammonium carminate and indigo carmin. The significance of this will be discussed later on.

Both *Molgula* and *Ascidia* were treated with indigo carmin at Beaufort but the pyloric gland showed no reaction to them.

In *Styela*, however, indigo carmin in concentrations as shown in the table gave in every case a characteristic reaction. The material in question were rather small *Styela* 20–30 mm. The large ones required too large receptacles.

Experiment	1.	100 c.c. saturated sol. of indigo carmin in sea water.	100 c.c. sea water		4 days.
"	2	100 c.c. do.	100 c.c.	do	2 "
"	3	100 c.c. do	100 c.c.	do	4 "
"	4	200 c.c. do			2 "

The animals in experiments 1, 2 and 3 lived, in experiment 4 they died.

When the animals were examined large blue concretions were found in the ampullæ and ducts of the pyloric gland (Figs. 25 and 26). These concretions gave that portion of the intestine covered by the pyloric gland a blue color. The writer considers that the indigo carmin was excreted from the blood into the canals of the gland.

Discussion.

Turning now to the character of the pyloric gland in other tunicates, we may as well begin with the Larvacæ. Although this organ is absent in most of the genera, it seems to be represented at least in a rudimentary form in two described by Chun ('81) from deep water of the Mediterranean Sea—*Stegasoma* and *Megalocercus*. Here we have a diverticulum of the gut which may be a possible homologue of the pyloric gland or at least to the pyloric cœcum. However, in no case is the organ developed as it is in the other orders of tunicates.

Our present knowledge of the pyloric gland in the Thaliacea and Ascidiacea can best be presented in tabular form. The table in question does not pretend to be complete but gives in condensed form the observations of various investigators.

Family.	Genus.	Authority.	No. of Ducts.	Type.	
Doliolidæ	*Doliolum*	Uljanin ('84)	1	Dendritic	Ciliated.
Salpidæ	*Salpa*	Chandelon ('75)	2	Reticular	——
Pyrosomidæ	*Pyrosoma*	Huxley ('59)	1	"Ramifying"	——
		Seeliger ('89)	1	Dendritic	——
Polyclinidæ	*Fragaroides*	Maurice ('88)	1	Reticular	Unciliated.
	Amaroecium	Author	—	——	Ciliated.
Distomidæ	*Distaplia*	Della Valle ('82)	1	Reticular	——
Botryllidæ	*Botryllus*	Della Valle ('82)	1	Dendritic	Ciliated.
		Pizon ('93)	1	Dendritic	Unciliated.
		Author	1	Dendritic	Ciliated.
Polystyelidæ	*Goodsiria*	Ritter ('96)	1	Branching	——
Clavelinidæ	*Clavelina*	Seeliger ('82)	1	Dendritic	——
		Author	1	Dendritic	Ciliated.
Perophoridæ	*Perophora*	Chandelon ('75)	1	Dendritic	Ciliated.
		Author	1	Dendritic	Ciliated.
Phallusidæ	*Phallusia scabra*	v. Winiwarter ('96)	2	Reticular	——
	Ascidia sp.	Author	—	——	Ciliated.
	Corella	v. Winiwarter ('96)	5–11	Reticular	——
Cynthiadæ	*Microcosmus*	Isert ('03)	1	Reticular	Ciliated.
	Polycarpa	Lacaze-Duthiers ('89)	1	Dendritic	Unciliated.
	Styela rustica	Wagner ('85)	1	Reticular	——
	Styela plicata	Author	1	——	Ciliated
	Styelina	Lacaze-Duthiers ('89)	1	Dendritic	——
Mulgulidæ	*Molgula* sp.	Author	—	——	Ciliated.

As far as this organ has been particularly described it is much the same in all families. The usual number of the ducts is one, but there may be more. The tubules are either dendritic or

form a network over the intestine. All the tubes, ducts and ampullæ that have been examined carefully have been found to be lined with a ciliated epithelium. Moreover, it is worthy of note, perhaps, that Huxley, ('51) and Della Valle ('81) have observed a bladder-like swelling of the duct in *Didemnum*. Todaro figures the same for *Salpa*. A type of gland differing slightly from the ones referred to above was described by Julin ('04) in *Archiascidia*. The single duct was short, and branched into but six tubules which did not branch on the intestine but ran parallel to one another almost all the way to the anus. There were no typical ampullæ. These tubes can best be compared to the rectal tubules described above for *Botryllus*.

As the writer mentioned in the introductory paragraph great difference of opinion exists as to the function of this organ. To be sure, few authors have ventured to strongly support one idea and most have been quite reserved in their conclusions, yet it is of sufficient interest to warrant the writer's reviewing their opinions briefly. Not considering those views that were based clearly on misconceptions cf. Vogt ('54), as to the structure of the organ, we will take up a few of the others. Really when Hancock ('66) called the organ in question a true liver much can be said to support the view. Its relation to the blood supply plainly recalls that of the vertebrate liver. Indeed with what we know at present of the organ, it would be very difficult to refute this idea, particularly as the vertebrate liver has been known to excrete carmin.

Chandelon ('75), Della Valla ('85) and von Winiwarter ('96) and Isert ('03) consider, after thoroughly reviewing the subject, that the function is digestive. Henri ('03) has other than morphological evidence. He says, p. 765: "En faisant des macérations de cette glande pylorique, on obtient un liquide riche en amylase, il ne digére ni l'albumine, ni la fibrine; cette macération agit au contraire faiblement sur la gélatine. Cette glande contient donc bien des ferments digestifs. Les macérations des autres parties du corps de la *Salpe* donnent des résultats négatifs."

Kupffer ('52) and Roule ('84) by means of injections arrived at the conclusion that these tubes were part of the blood vascular system. Lister ('34), Huxley ('51) and after them Pizon ('93)

and Lefevre ('98) have agreed that it probably serves an absorbing function, something like the gastrovascular canals of Cœlenterata. Huxley ('51) asked: "Does this tubular system represent a hepatic organ or is it not more probably a sort of rudimentary lacteal system—a means of straining off the nutritive juices from the stomach into the blood by which these tubes are bathed?" It is very probable that the organ has a digestive function; there seems strong evidence to support that idea. But the direction of the cilia in the duct would forbid the conclusion of Huxley, etc., that the function is that of absorption.

There is yet another function that has been attributed to the pyloric gland. Kowalewsky ('74) was inclined from what he knew of the structure of the organ in *Peropora* to attribute to it urinary functions.

Krunkenberg ('80) says: "Ich finde sie als constantes, durch die Murexideprobe leicht und schön nachzuweisendes Product der als Nieren angesprochnen drüsigen Darmanhänge bei *Phallusia mentula*." This statement is based on a misconception. He did not distinguish that the pyloric gland and the renal vesicles were not part of one system. What he analyzed were the concretions which others have found to contain uric acid. This interpretation is supported by the fact that he could not find uric acid in the gland of *Ciona* and of *Cynthia*, neither of which have renal vesicles covering the intestine.

In *Salpa*, Todaro ('01–'02) described three pairs of diverticula from the alimentary canal that had the power of taking up carmin. The first pair was in the pharynx, the second pair in the œsophagus and the third pair the pyloric gland.

Let us now turn and inquire as to what organs have been previously described as possessing the power of elimination of waste products of metabolism from the body of tunicates. Roule ('84) makes the distinction between a kidney of excretion and one of accumulation. Through the investigations of Van Beneden ('46), Kupffer ('72), Lacaze-Duthiers ('74), Kowalevsky ('89) and Dahlgrün ('00), we have a knowledge of this latter type of organ at least in a few groups. The kidney accumulation may be said to consist of two types. In *Salpa*, *Ciona* and *Botryllus* it consists of blood cells containing brownish concretions. The second type

is composed of closed vesicles lined by an nonciliated epithelium which encloses a fluid in which are suspended one or more relatively large concretions. There may be many small vesicles as in *Ascidia* and *Ascidiella*. In *Cynthia* we have a few larger vesicles and in *Molgula* a single large one.

In a kidney of accumulation, the waste matter of the organism is stored up in the form of a solid which is freed from the organism only by death. Harmer ('92) has described such an organ in the ectoprocta and the kidney of accumulation can be found in several groups of animals. In the Tunicata other organs have been described as kidneys of excretion. Julin ('91) suggested that the neural gland had perhaps an excretory function. Metcalf ('00), on reviewing the subject of the neural gland, considers that there is no evidence to support the view of Julin. Roule ('84–'85) on the other hand, described about the opening of the deferent canal in *Ciona* a mass of pigment cells which according to this author is a kidney of excretion.

To the view that the pyloric gland is a kidney, there is one serious objection. This is the fact that Henri ('03) found in the pyloric gland of *Salpa* a diastatic ferment in great abundance. This would seem to be a strong argument in favor of a digestive function for the organ were it not that such a ferment is found in the kidneys of certain mammals, sparingly in the dog it is true but richly in the rabbit (Oppenheimer, '09).

We have in certain forms of tunicates a kidney of accumulation, but in nearly all groups a pyloric gland. The Appendiculariæ which are without it are so exceedingly minute that all their tissues, if not actually bathed by the sea water, are in close proximity to it, so that the need of special organs of excretion is not quite so urgent. Be that as it may the question naturally arises, has this tunicate organ any characters in common with the excretory organs of other groups of animals?

The shape and character of the terminal bulbs, and the ducts are parallelled in part by the multicellular ciliary flames found in the Nemertinea. A section of the terminal end of a duct in *Lineus* by Punnett ('11) shows a condition very similar to that of the ascidian. Each cell of the organ bears a single flagellum which is directed away from the blind end of the tube. That the

duct of the pyloric gland opens into the stomach and is derived in development from the entoderm is a condition characterizing no other excretory system out side of the Arthopods. However, since the vertebrate liver has been shown by Chrzonszezewsky ('66) to excrete carmin, and has morphologically the same position as has the pyloric gland of the ascidian and has a similar development, there is good reason to believe that the two are homologous. Willey has homologized the organ with the hepatic cœcum of *Amphioxus* which Hammar ('93) about the same time compared to the liver of the Craniota. Can we not conceive that in the hypothetical ancestor of the vertebrate the liver arose as an organ of excretion and in the tunicate it has retained more of those characters?

Summary.

1. There are in *Botryllus* two sorts of terminations to the tubes that compose the pyloric gland, bladder-like ampullæ and long straight blind tubes—the latter we have called rectal tubules because in many cases they extend to the region of the rectum.

2. The ducts and ampullæ of *Botryllus* as well as *Ascidia*, *Styela*, *Molgula*, *Perophora*, *Clavelina* and *Amaroecium* are lined by cells bearing long whip-like flagella, the ends of which are directed toward the mouth of the duct.

3. Many of the rectal tubules have a termination difficult to interpret. This has the appearance, in most cases, of a cup-like depression in the end of the tube which seems to form a communication between the blood cavity and the lumen of the tube. In no case, however, could such a communication be demonstrated.

4. The direction in which the free ends of the flagella point indicates that the contents of the lumen pass toward the stomach and therefore the function of the organ is secretory rather than that of absorption.

5. Part of this secretion is probably found in the minute yellow globules found in the cells of the ducts and ampullæ. If these yellow globules represent a secretion, this is soluble in water and does not form masses in the lumen of the tube as in *Microcosmus* and *Styela*.

6. Bismarck brown and neutral red are concentrated in the

lumen of the organ in the form of a liquid while the indigo carmin is found concentrated in solid form in the gland of *Styela*.

7. In the tunicates in general no special kidney of excretion has been recognized. Although the gland in question may have other functions also, yet its structure and properties seem to indicate that it is the kidney of excretion of the tunicates, and is in turn homologous to the vertebrate liver.

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Description of Plates.

The figures were all drawn with a Zeiss microscope and apocromatic lenses. In making all the drawings a camera lucida and artificial light was used, in the case of *Botryllus* a Welsbach burner, and in the case of the other forms an ordinary oil lamp. With the exception of Fig. 1, Fig. 16 and Fig. 17 all were drawn at a magnification of 1,500 diameters, and have been reduced in reproduction to 1,000.

A = ampullæ.
bc = blood cell.
C = concretions of indigo carmin.
I = intestine.
Nu = nucleus.
O = Œsophagus.
PC = pyloric cœcum.
PS = peribranchial sac.
R rectum.
RT = rectal tubules.
RO = pyloric gland.
S = stomach.
V = vacuole in intestinal cell.

Explanation of Plate I.

Fig. 1. The stomach and intestine of *Botryllus*. × 52.

Fig. 2. Two ampullæ of the pyloric gland in *Botryllus*. This drawing shows the long whip-like flagella and also the granules in the cells. × 1,000.

Fig. 3. Portion of one of the main ducts. × 1,000.

Figs. 4–7. Optical section of a rectal tubule showing segmentation, granules, and flagella. × 1,000.

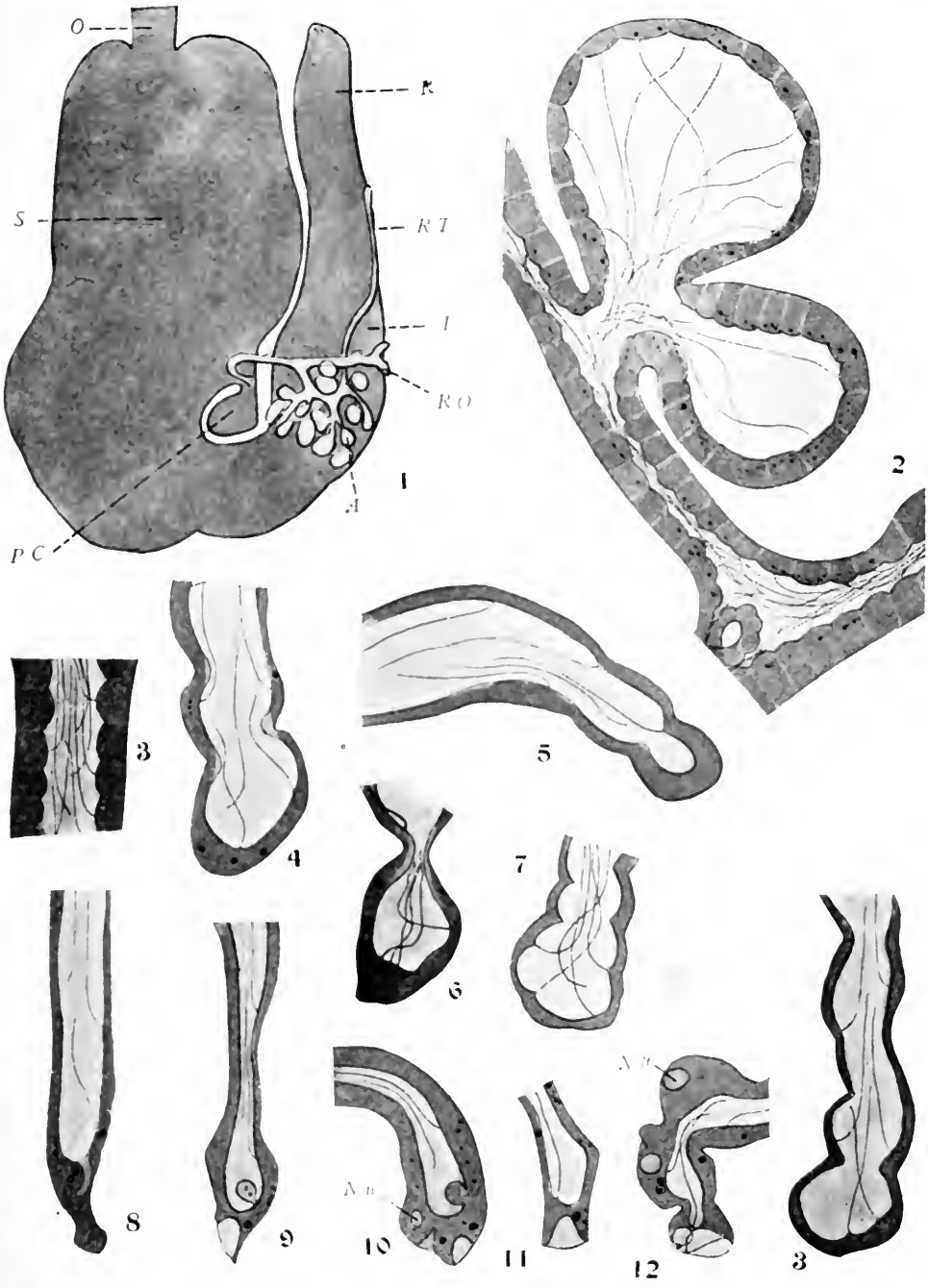
Fig. 8. Optical section of a termination of a rectal tubule showing a thin place in the wall of the tube. × 1,000.

Fig. 9. Optical section of a cup-like depression in the end of a tubule. × 1,000.

Fig. 10. Optical section of a tube with two cup-like depressions at the end. × 1,000

Figs. 11–12. Termination with a single cup-like depression. × 1,000.

Fig. 13. Optical section of a segmented tube in the walls of which there are no granules. × 1,000.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Explanation of Plate II.

Fig. 14. Section 6 μ thick of the termination of a rectal tubule of *Botryllus* which was fixed in Flemming's solution. This shows a thin place in the wall of the tube and also a vacuole. In this figure is also shown the relation of the tube to the walls of the intestine. The vacuoles in the intestinal cells are those which stained red and blue in ammonium carminate and in indigo carmin respectively. × 1,000.

Fig. 15. Drawing made from two adjoining sections of a rectal tubule, which show terminations with both a thin place and a depression. × 1,000.

Fig. 16. A section of a bud of *Botryllus* showing the origin of the pyloric gland as an out pocket to the stomach. × 320.

Fig. 17. Sections of the rectum of *Botryllus* fixed in absolute alcohol, glacial acetic acid and chloroform. This shows the relation of the tubes to the canal. × 150.

Fig. 18. Two living blood cells, in one of which the nucleus is visible in the other it is not. × 1,000.

Fig. 19. Optical section of ampulla of *Ascidia*. × 1,000.

Fig. 20. Optical section of rectal tubule of *Ascidia*. × 1,000.

Fig. 21. Optical section of ampulla of *Perophora*. × 1,000.

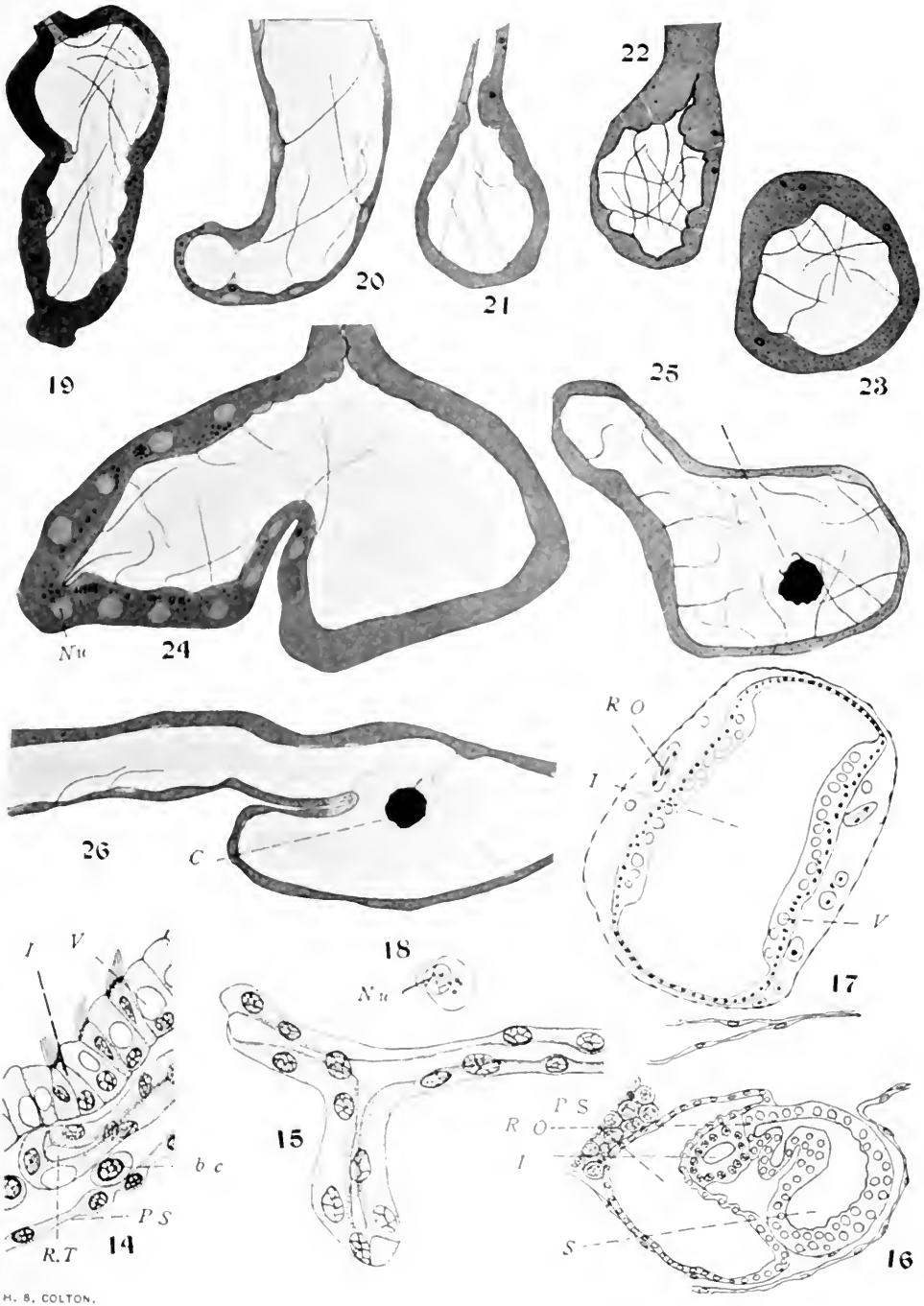
Fig. 22. Optical section of ampulla of *Perophora*. × 1,000.

Fig. 23. Optical section of ampulla of *Molgula*. × 1,000.

Fig. 24. Optical section of ampulla of *Styela*. × 1,000.

Fig. 25. Optical section of ampulla of *Styela*, containing a concretion of indigo carmin. × 1,000.

Fig. 26. Optical section of a tube of the refringent organ of *Styela* containing a concretion of indigo carmin. × 1,000.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THE SPERMATOGENESIS OF THE CADDIS-FLY (PLATYPHYLAX DESIGNATUS WALKER).

B. F. LUTMAN.

The numerous works that have appeared in recent years on the spermatogenesis of insects have covered practically every order and in some orders, such as the Hemiptera, many of the important families. There still remain, however, a number of the smaller orders which have not been investigated, and there is always the possibility that these may contain new or especially favorable structures. The Trichoptera is one of these small orders. The Neuroptera and Lepidoptera to which it is believed to be most nearly related have been investigated by Miss McGill (8) and by Toyama (15) and Henking (2). The small size of the larvæ and the difficulty of dissecting out the very young tests has probably deterred investigators from attacking this group when larger, better known and more easily obtainable forms were to be had in the Hemiptera and Orthoptera.

I shall not go into an extensive discussion here of the general literature of spermatogenesis. *Platyphylax* has in the main the same development that has been described for all insect forms. There are certain points in which it is different and the literature on them will be discussed in connection with my own observations.

The only paper in which the spermatogenesis of the Trichoptera is discussed at all is in that of Lubben (3). Lubben was more interested in the external morphology and the development of the testes than he was in the cytological details, but he observed a number of facts well worth noticing. The spermagonial cells arise out of the original genital cells by division. They grow into large cells which appear, united with two cyst epithelial cells. The origin of these epithelial cells of the cysts was not clear to him. Inside these epithelial cells now arises a mass of cells by division. Then by two more divisions the spermatids are produced; these have sharply bounded nuclei with clear plasma around them. The spermatids increase in length and the whole

complex of spermatozoa takes on the form of a long-drawn out cylinder. The nuclei are all arranged at one end, pointing one way, while the body of the sperm fills the remaining space. These he calls spermatocysts.

The formation of the free spermatozoids does not seem to occur either in the free or the enclosed larvæ. At the time of pupation there are only spermatocysts. In the pupa there results along with growth and the further differentiation of the spermatozoids the resorption of the walls of the spermatocysts. In the mature pupa the spermatozoa are free and lie in thick knots in each division of the testes.

The caddis-flies, as has long been known, pass practically their entire life in the larval and pupal stage. Vorheis (17), who has followed the life-history of *Platyphylax designatus*, has found that the eggs are laid in April and that the larvæ appear about two weeks later. During summer and fall the larvæ grow, and from November to January more and more larvæ are found. The period of pupation begins about the middle of February and is indicated by larger irregular stones being attached to the anterior end of the sand cases "while some are attached to the lower surface of the large rocks by a mass of silk at the anterior end." For a few days after the closing of the case the larvæ remain inactive but unchanged, before becoming pupæ.

The caddis-fly larvæ offer the advantage that while the life-history just sketched is gone through with at about the time indicated, larvæ of almost any size may be still obtained up until January and February. The material was obtained during three years; the first year from about the first of December to the first of February; the second year during the month of June, and the third year in May. The spermagonial and reduction division occur in the larval stage, so the half-grown larvæ of a length of 6–10 mm. was the material in which it was found. The sperms are apparently all formed at or soon after pupation.

In the older specimens the testes were dissected out, but in order to get the very young spermagonia it was necessary to section the entire abdomen.

Practically all the material was fixed in Fleming's weaker solution although sublimate-acetic was used on some abdomens

with very good results. The sections were stained in Heidenhain's iron alum hæmatoxylin or in Flemming's triple stain.

The Testes.

The testes, as Vorheis (17) has already noted, occur in the fourth and fifth segment of the abdomen. They are small five lobed structures usually surrounded by a mass of fat.

These five lobes or follicles are about equal size but of various shapes. In general they are somewhat wedge-shaped, the thinner part of the wedge being, of course, toward the point of attachment of the organ (Fig. 13). In longitudinal section about fifty cysts appear in each follicle (Fig. 14), the larger ones at its smaller end.

Each follicle has its own wall and the flattened nuclei of these wall cells frequently appear in the sections. In addition each cyst is provided with a wall. The youngest cells are farthest from the point of attachment of the testes. In one section, as shown in Fig. 14, cysts containing spiremes for the first division, tetrads, first and second divisions, transformations of the spermatids, and fully developed sperms occur in the order named across the section. The young cysts show in section, 6–8 spermatocytes; the older ones, where the cells are smaller, due to the two divisions and to the growth of the cysts, show 12–18. There is no definite zonation of spiremes, first divisions, second divisions, etc., such as Wilcox (19) has figured in *Caloptemus femur-rubrum*, but the general tendency is for the cysts containing the younger cells to be further away from the smaller end of the follicles. The fact that there is no definite zonation of successively older spermatocytes makes it at first confusing in picking out the sequence of development. The difference in size and number of them in the different cysts however makes this possible after a little observation. The spermatids in developing in the cysts, as is usual, have their heads all pointing in one direction as they lie side by side.

The Spermagonial Cells.

As the early history of the spermagonial cells does not seem to have been worked out on many species of insects a rather full description of that process will be given here. In this insect the

primary and secondary spermagonial cells are sharply distinguished in their division stages while the development of the cysts with their surrounding epithelial cells is almost diagrammatic in its clearness.

There seems to be a general agreement in the literature that each of the cysts that lie in the follicles of the testes has its origin from a single germ-cell. This conception seems to have originated in the work of Vallette St. George (12) on the testes of *Rana temporaria*. He found that if he teased apart the parenchyma of the testes, groups of cells, that he called spermatocysts, would drop out. These structures had walls of their own in which were imbedded one or two nuclei. St. George believed that each of these cysts arose from a single cell, one of his "Ursamenzellen." These would correspond to the last of what is now known as the primary spermagonial cells. By the division of these cells there was produced the spermacysts containing the spermagonia; or as now called, the secondary spermagonia.

Montgomery (10) describes the cysts in *Pentatoma* but did not work out their origin. The connective tissue network of the young testes contains, he believes, in each mesh a single spermagonium or at least only a few spermagonia. These cells divide and the cells produced by their division are surrounded by a cyst-membrane derived from an extension of the connective tissue investment of the follicle. The germ-cells and the cells of the cyst walls have then a different origin.

Several others, among them Henking, Paulmier and McGregor, are mentioned by Sutton (14) as having noted the arrangement into cysts and the cyst walls but none of them seems to have followed their development.

Sutton (14) in studying the spermagonial divisions in *Brachystola magna* also worked out fairly completely the development of the cysts. A cyst membrane with nuclei in it is formed even in the two-celled stage of the secondary spermagonia. The cysts assume a roughly pyramidal shape, the cells inside it largely dividing tangentially to the surface of the cyst. All the cells in one cyst seem to divide simultaneously producing by means of about eight divisions, 256 cells. The cysts are not attached to the walls of the follicles.

In all of the species so far described, however, the cysts lie so closely appressed against each other and against the follicular wall that it is impossible to decide certainly as to the identity of the cyst walls. In all of them too the number of cells seems to be large. In these respects *Platyphylax* offers a much better opportunity for deciding the question of cyst wall and cell number in a cyst.

The earliest stage of the reproductive organ that I was able to be sure was the testes was that shown in Fig. 1. At this stage there has already appeared the lobing into five divisions that is so characteristic of the mature organ. The cells composing it are apparently all of about the same size; there being at this time no visible differentiation into germ-cells and cyst epithelial cells, if such does occur. The nuclei of these cells are a little larger than those in other parts of the body. The distribution of chromatin in these nuclei is characteristic of that in all parts of the body, the pieces being of rather large size and of a rather regular number instead of having the form of fine granules. Sometimes these pieces of chromatin are in the form of bodies which might well be taken for split chromosomes, while others have the diamond shape characteristic of the tetrads with which they might readily be confounded (Fig. 3). Divisions are rather rare at this time and growth is apparently rather slow. The division figures observed were of typical mitotic type. The peculiar part that the nucleolus plays in this division will be discussed under the reduction divisions as in them it is much larger and more readily followed. All that is necessary to say here is that it seems to change its spherical shape, becomes elongated and apparently forms a chromosome.

In the testes next advanced in the stage of development the primary spermagonial cells occupy only about a third of the space while the remainder of it is filled with the secondary spermagonial cells in various stages of division to form the mature cysts can be followed at every stage.

The testes have grown considerably. The five divisions are fully formed; they have acquired distinct cellular boundaries; and the original secondary spermagonial cells, instead of being rather closely packed in the testes, are now lying with consider-

able free space between them. This free space is what makes the task of following them, and their boundaries, so easy. About two thirds of the primary cells, spermagonia, or "Ursamenzellen" as they are variously called, have divided to form groups of 2, 4, 8, 16 or 32 cells while the other third is undivided.

A closer examination of these undivided cells will now show that in addition to the larger germ-cell there is lying closely appressed against it a smaller kidney-shaped nucleus (Fig. 4). The chromatin in both nuclei is distributed in the form of little flecks rather regular in size and number and united by strands.

The origin of this nucleus that lies in the epithelium of the cyst is not clear. There is no such differentiation in the cells in the testes at the preceding stage. If these epithelial cells were in the testes at that time it was impossible to distinguish them. It may be that it is an epithelial cell that has made its way in some fashion into the interior of the testes and there surrounded the germ-cell. It is a difficult question to decide and one on which my material gives little evidence.

The further development of the cysts of the follicle can be readily followed in the two- (Fig. 5), four- (Fig. 6), eight- (Fig. 7), sixteen- and thirty-two-celled (Fig. 8) stages. The cell-walls are difficult to distinguish at these stages as the plasma membrane which is all that surrounds the cells is very thin and the cells are pressed tightly against each other. The same difficulty was noted by St. George (12) who found, however, that he could distinguish the walls in material fixed in "quick acting reagents." At any of these stages the nucleus of the cyst epithelium will show. In the older cysts there appear quite frequently to be two such nuclei, but in the younger stages at least I have observed only one. These nuclei do not seem to divide.

The divisions in the secondary spermagonial cells are very regular, all the cells in one follicle dividing at one time (Fig. 9). All these divisions are typically mitotic. Sutton (14) found that the division spindles were largely tangential to the walls of the cyst, owing to pressure in those directions. This caused the cyst to grow in length. As the cysts of the caddis-fly are spherical, the divisions occur in equal numbers in all planes. It can be readily determined that there are regularily 32 cells in each cyst of the

follicles, which indicates, of course, that five divisions have occurred.

After the last division, the cells of these mature cysts grow and the nucleus becomes much larger in proportion to the size of the cell. Fig. 8 shows the size of the cyst immediately after it has acquired its full number of cells and Fig. 10 shows a cyst after the growth period in which the cells are in the spireme for the reduction divisions. There are therefore two growth periods: one after the last primary spermagonial division and the second before the reduction divisions.

The Reduction Divisions.

After the last spermagonial divisions the growth is apparently very rapid. The nucleus is very large, occupying almost the entire cell and the chromatin is in the form of regular little pieces (Fig. 11). The remains of the spindle of the last spermagonial division lies near the nucleus in the form of an ovoid body, the nebenkern.

Apparently the first stage in division shows the little pieces of chromatin drawn out until they resemble chromosomes while the nucleolus becomes oval or spindle-shaped (Fig. 12). The pieces of chromatin become united by a slender connection and seem to spin out and lose their identity. The nucleolus in the meantime becomes more and more elongated (Fig. 15).

The chromatin now seems to go into a long slender spireme, the mass of the threads lying at one side of the nucleus and occupying about one half of it. This is apparently the stage of synapsis. The strands are very fine and delicate and as the nuclei are not so very large, it is impossible to make out any pairing of the threads, if such occurs. The nucleolus lies as a long drawn out body either in this tangle of the threads or on its surface. Occasional loops stick up out of the denser clump but they are too small to follow or to make much out of. After these stages, which are apparently short as they are not numerous, the chromatin again fills the entire cell in the form of the spireme. This spireme gradually becomes shorter and thicker. This stage is a very long one as is shown by the fact that in sections where any divisions at all occur, about half of the cells are in this stage.

It may be that the stage of synapsis is much shortened in the caddis-fly and that of the spireme is correspondingly lengthened.

The spireme now breaks up into chromosomes and these lie in the nucleus as long slender paired bodies (Fig. 22). The chromosomes now come to have the peculiar shapes such as X's, Y's, twisted figures, etc., characteristic of the stage of the reduction division (Fig. 23). These soon shorten into the tetrads (Fig. 24). The tetrads take on the typical lozenge form and sometimes show an opening in their center. The manner in which these bodies arrange themselves on the spindle could not be definitely determined as they are small in size and the four segments of the lozenge are all of about the same length.

The metaphase shows a sharp pointed spindle with its extremities in a centrosome just inside the plasma membrane (Fig. 25). The telophase of this division shows the chromosomes pulled about two-thirds of the way back to the centrosome and the nuclei still connected by the remains of the central spindle (Fig. 26). The centrosome at this time is still apparently divided and the rays from it extend down to the cluster of chromosomes.

The second division, following almost immediately, has little to distinguish it in the metaphase from the first except in its size and in the size of its chromosomes (Fig. 29). In the telophase of this division the centrosome was not to be found—just what had become of it has not been ascertained. The remains of the central spindle is the most conspicuous feature of this stage. The chromosomes seem to spread out at once, as soon as a nuclear membrane is formed, and make the ordinary reticulated network of a resting nucleus (Fig. 34).

There is a period of growth after the reconstruction of the nucleus, such as Paulmier (11) and other authors have described, followed by a stage in which the nucleus shrinks. Even before the chromosomes are entirely distributed and while they are still present as little pieces of chromatin (Fig. 34), the cell begins to lengthen and the axial filament to form. In fact the long drawn out form of the cell which it has from the last division does not seem to change but passes over at once to the young sperm. The transition stage takes a long time for its completion and any number of transition stages can be found. The chromatin col-

lects more or less to the outside of the nucleus, forming a hollow sphere, just inside the nuclear wall. The remains of the spindle lie near the nucleus as an oval body in a clear zone. This body apparently divides as Baumgartner (1) has described and a part of it seems to pass around the nucleus. Soon after these stages shown in Fig. 34 the nuclei become very sensitive to the fixing reagents and as a consequence practically all of them have collapsed. This would probably be the stages when the chromatin is in the form of a tube with a very small lumen. The fully developed sperm is shown in Fig. 36.

The details given above are in the main the same as have been described many times for animal spermatogenesis. In the action of some parts of the cell mechanism there is always quite a little variation and it is to these parts that particular attention will be paid. The two structures to be especially noted are the centrosome and the "chromatin nucleolus."

The Centrosome.

It is impossible to locate the centrosome except when it is occupying a position at the end of the spindle. At any other place it is impossible to say whether a certain dark staining body is a centrosome or not. Bodies appear near the nucleus where the centrosome should be and have all the characteristics of centrosomes, being darkly staining little granules surrounded by a clear space and frequently with what appear to be rays running up to them, though these latter are very faint and small, but the multiplicity of such bodies makes it impossible to trace the history of the centrosome with any certainty for any distance or even to be sure that such a centre is present at all times. At the ends of the spindle, its position is of course always easy to determine. It usually first appears so as to be definitely recognized at the tetrad stage as a dark body on the plasma membrane. It appears as a very small black granule on which the fibres terminate and lies just inside the plasma membrane and apparently attached to it. It is most conspicuous during the metaphase when the sharp pointed spindle ends in this little granule at the plasma membrane. It may be, of course, that here it is not a body at all but only the common point of attachment of the fibres of the

central spindle and of the aster. Whatever the origin and nature of the centrosomes may be at this time it is at least something that will take a stain and that has a definite location. After the second division, at which time it lies at the ends of the spindle again, it seems to disappear until a dark staining granule appears at one side of the nucleus from which the axial filament seems to be growing out. I have not traced the centrosome around to discover whether the two are identical or not but from the results on other animals it undoubtedly is. From this stage on then, it would form the middle piece of the sperm.

The Chromatin Nucleolus.

McClung (4) has described in the germ-cells of certain grasshoppers a body which he calls the accessory chromosome. Previous to this discovery of this body the "chromatin nucleolus" had been described by Montgomery (10) in the Hemiptera. More recently the discussion of heterotypic chromosomes has been given special importance by the papers of Stevens (13) and Wilson (20, 21) especially in connection with the theory of sex-determinants.

The divisions in the nuclei of *Platyphylax* show a body which, while it seems to have something in common with these described structures, is in other respects quite different. Its behavior has been reserved for this separate discussion.

The various changes undergone by this body have been followed to some extent both in the spermagonial and reduction divisions. As the cells, and consequently this chromatin nucleolus, are larger in the reductions divisions it will be described there first. The nucleus of the young spermatocytes contains a nucleolus that stains typically both in the triple and the iron haematoxylin. This body is either spherical or ovoid in shape. In the preparatory stages of division it begins to lengthen and become spindle shaped. It frequently lies twisted over on itself or is spoon-shaped at this time (Fig. 15).

In a later stage when the chromatin has gone into the synaptic condition this body seems as a rule to be somewhat smaller in diameter as though it were spun out as the other chromatin has been (Fig. 16). It does not lie among the chromatin strands, but

as a rule rests outside the chromatin mass which at this time occupies about half the nucleus. At this time the body seems to be a part of the spireme. In the next stage when the chromatin comes out of synapsis this body appears as a part of the much thickened spireme thread (Figs. 17–21). It is quite large at this time and much resembles a nucleolus in its intense staining reactions but it is spindle shaped and from each end runs out the continuation of the spireme thread. In the triple stain at this time it still takes the safranin color. A still closer examination shows that the threads leading up to this body are double and the body itself is divided into two halves by a longitudinal furrow. It lies at this time, when seen in side view, as a flat spindle-shaped figure immediately pressed against the nuclear wall. It now resembles very much the accessory chromosome as drawn by McClung (6) in his Fig. 2, except that he at this period discovered no break and the body that he drew was proportionally considerably larger. He describes and figures a stage (Fig. 5) where this body goes into a spireme of its own but no split was observed in this separate thread. Considering the subsequent behavior of this body—the formation of a tetrad and of chromosomes, such a split is to be expected. A split was to be observed in this chromatin nucleolus of *Platyphylax*; its spireme is a part of the spireme formed from the remainder of the chromatin. The split in it becomes more marked (Fig. 19) and the body finally opens out as a lozenge-shaped tetrad (Fig. 21). At this time the other chromosomes have not yet formed, although the longitudinal split has taken place. In some cases it looks as though the transverse splits have already occurred, but the thread still remains intact with this body as a part of it. This black staining tetrad is one of the most conspicuous parts of the nucleus at this time (Fig. 21).

The other chromosomes are now formed and assume the peculiar shapes characteristic of them at the time before they form the tetrads. This body is still recognizable at this time on account of its regular lozenge shape while the others are in the form of X's, Y's and various other twisted shapes (Fig. 23). At the next stage, however, when all the chromosomes have become tetrads this body is indistinguishable from them (Fig. 24).

There is no evidence that it has disappeared as the nucleolus usually does; it seems simply to have become a tetrad. The elaborate formation and dividing of the tetrad would argue against this disappearance also. This chromatin nucleolus can be traced no further. During the equatorial plate stage of division the chromosomes all lie in one plane and it is impossible to identify any particular one as the transformed nucleolus. Neither does any one lag behind in divisions in the metaphase nor in the movement toward the poles in anaphase (Figs. 26 and 32). If this body forms a chromosome, as it undoubtedly does, that chromosome behaves exactly like all the others.

The number of chromosomes is of great interest here if this is a true accessory chromosome. According to either the McClung or the Wilson type of an accessory chromosome, or the Wilson type of a heterotypic one, there should appear an odd number of chromosomes plus this additional one; or as McClung has found in *Orchesticus* sixteen chromosomes and the accessory one.

In all the counts made in *Platyphylax*, however, the number of chromosomes for both the reduction divisions was found to be thirty. This is the result of repeated trials. These countings are as easy to make as of dots on a piece of paper (Figs. 27, 28, 30) as the polar plate views are numerous and the chromosomes are short. There is some variation in size in the chromosomes in polar view but it is impossible to pick out one of them as the special structure that has been followed.[1]

It will be seen from this description that while this body resembles the accessory chromosome of McClung in many respects still it differs from it in one very essential one. It apparently forms a tetrad that divides in both divisions and so each sperm would receive one fourth of it. This would make it impossible for it to serve as a sex-determinant, for all the sperms would receive a part of it, and not half of them, as would happen if we credit the observations of McClung or of Miss Wallace (18). This body in that case could not be a sex-determinant.

[1]In cutting abdomens to get the development of the testes I have cut and stained as many, or more, females than I have males. The divisions in the former can be readily observed here as they are much larger than in the testes. The nucleolus undergoes a similar lengthening out, and then forms a part of the spireme. Marshall (9) in his paper on the development of the ovary also shows several figures that strongly suggest this.

Voinov (16) has described in the divisions preceding sperm formation in a beetle (*Cybister roeselii*) a body which very much resembles in its appearance the one under discussion. He, however, did not observe it forming a chromosome tetrad, nor did he follow it during the nuclear divisions except to figure a small darkly staining body lying outside the nucleus in the cytoplasm, which he believes to be the same. It may be that this body might form a tetrad as in *Platyphylax*.

Heidenhain in "Plasma und Zelle" approves Flemming's opinion that the nucleolus is always surrounded by a thin layer of true chromatin. Some appearance such as those shown in Fig. 2 would seem to suggest at least that there might be a ring of some other material around the nucleolus but the structure is so small that it is impossible to say certainly. If this were true, however, the changes described would only mean that the nucleolus loses its form during the divisions and becomes pulled out and split, between the chromatin strands surrounding it.[1]

At any rate whatever this structure in *Platyphylax* may be, whether "accessory chromosome" or "chromatin nucleolus," some disposition of it must be made in discussing these aberrant chromosomes in the nuclei of insects.

This work was done under the direction of Prof. W. S. Marshall, of the University of Wisconsin, to whom my thanks are due not only for assistance with methods and literature but also for quite a portion of the material from which the sections were made.

Summary.

1. The development of the follicular cysts can be readily followed in this insect. Each cyst contains 32 cells derived by 5 divisions from a primary spermagonial cell and enclosed in a membrane containing one or two nuclei.

2. The reduced chromosome number is always 30; the somatic number is probably 60 from a count in the oogonial divisions.

3. The centrosome is only to be followed from the tetrad stage to the anaphase but probably forms the middle piece of the sperms.

[1] A count of the somatic number of chromosomes in the oögonial division gave 55–60. The exceedingly large number makes counting difficult and not very accurate in these divisions.

4. The nucleolus of the spermatocyte seems to form a tetrad which becomes one of the thirty of the reduced number.

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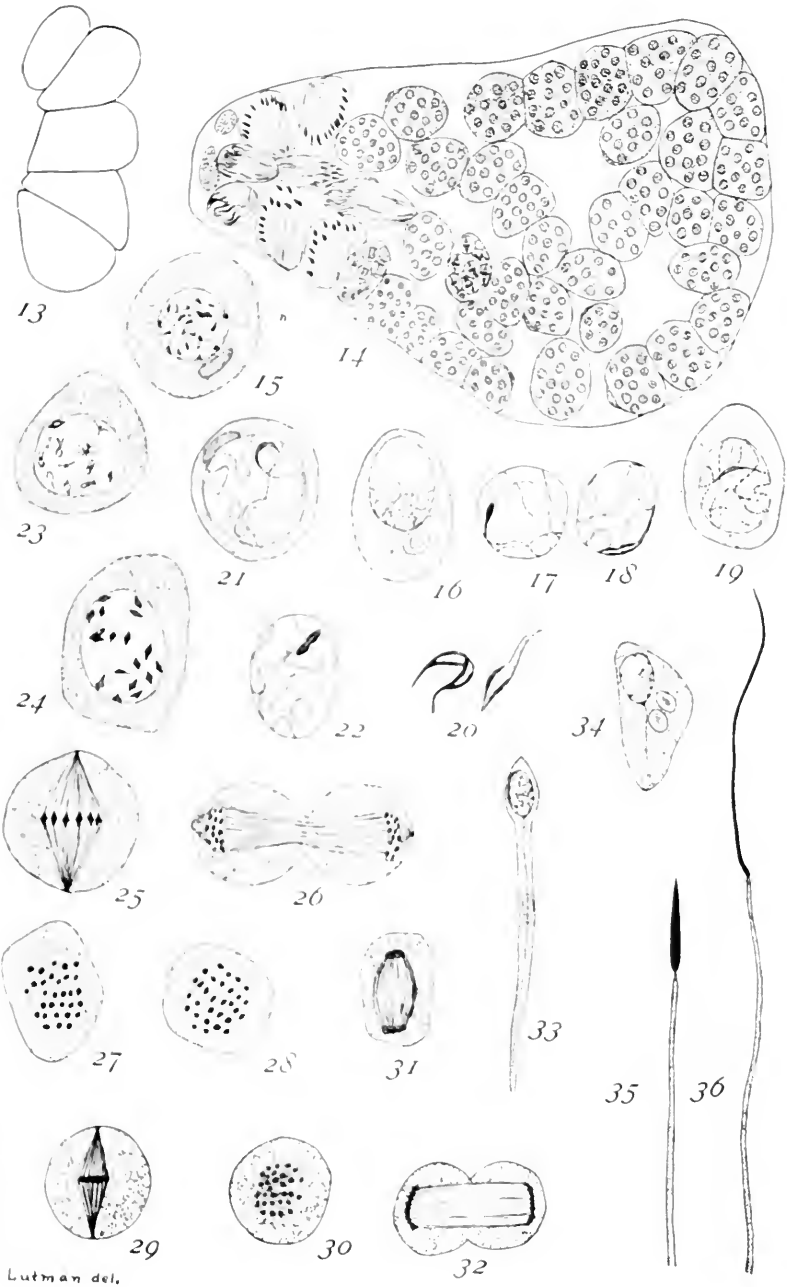
Explanation of Plate II.

13. Longitudinal diagram of the entire mature testes.
14. Section of one of the follicles showing size, shape, and distribution of the cysts.
15. Formation of the spireme in a spermatocyte.
16. Synapsis.
17. Nucleus showing nucleolus on spireme.
18. Nucleus showing nucleolus on spireme.
19. Entire cell showing same.
20. Enlarged views of the nucleolus at this same stage showing the split.
21. Chromosomes formed by the transverse split.
22. Chromosomes lying free in the nucleus; also the split nucleolus.
23. Chromosomes forming the tetrads; nucleolus at *n*.
24. Tetrads.
25. Metaphase, first division.
26. Anaphase, first division.
27–28. Polar view of the equatorial plate.
29. Metaphase, second division.
30. Polar view, equatorial plate.
31–32. Anaphase, second division.
33–35. Transformation stages of the spermatid.
36. Sperm.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BIOLOGICAL BULLETIN

THE ASSOCIATION OF A FISH WITH A HYDROID.

HAROLD HEATH.

Among the most interesting phases of animal existence are those examples of commensalism or "messmateism" known in many instances to exist among species of widely different phyla. At times the association, while no doubt beneficial to both parties, is purely accidental, such as that occurring occasionally between the colonies of various hydroids and crabs or molluscs and on two occasions I have found flourishing colonies of *Clava leptostyla* attached to the spines of the sea urchin, *Strongylocentrotus franciscanus*. Again what appears to be a communistic association may in reality be a case of parasitism as, for example, the relation of certain hydroids and the eggs of a number of fishes, or possibly one hydroid to another as noted by Allman in his monograph on gymnoblastic hydroids, or the attachment, mentioned by Fewkes,[1] of the hydroid, *Hydrichthys mirus*, to the fish, *Seriola zonata*. In undoubted cases the association is not only invariable, or fairly constant, but mutually beneficial and very intimate as is witnessed by the fact that the hermit crab, *Eupagurus prideauxii*, when changing its abode removes the commensal anemone, or the case mentioned by Miss Rathbun[2] of the Hawaiian Island crab, *Lybia tesselata*, that held "little sea anemones one in each claw and presented them in a boxing attitude whenever teased or approached by another crab." And, on the other hand, it is generally believed that the anemones enjoy a larger food supply consequent upon the improved method of locomotion.

In 1892 Alcock[3] added what he considered to be another of

[1] *Proc. Boston Nat. Hist. Soc.*, Vol. XXIII.

[2] U. S. F. C. Bulletin, 1903, p. 866.

[3] *Ann. Mag. Nat. Hist.*, 6 ser., Vol. 10.

these remarkable mutual benefit societies, describing the association of the hydroid *Stylactis minoi* with the rock perch, *Minous inermis*. Several specimens were taken at depths varying from forty to seventy fathoms in the Bay of Bengal and the Laccadive or Malabar sea; and in every case the hydroid was found attached in large numbers about the gill opening, on the throat and in the axilla. And not only were no fish of this species ever discovered without being coated with the hydroid, but none of these hydroids was ever found upon the multitudes of other animals dredged in the same locality, though among these were specimens of *Minous coccineus*. Accordingly it thus appears to Alcock that, unusual though it is, this is a case of true commensalism.

Several years later Doflein collected three more specimens of *M. inermis* in Sagami Bay, Japan, and again all were coated, especially between the pectoral and ventral fins, with this same hydroid. In the first account the cœlenterate was assigned by Alcock, on characters associated with the reproductive sacs, to the genus *Podocoryne;* but other specimens, seemingly more highly developed, led the author later to place them in the genus *Stylactis*. On the other hand, Stechow,[1] who described the Japanese specimens, finds no evidence of sporosacs, but young medusæ with tentacles and four radial canals and accordingly this author places the species once more in the genus *Podocoryne*.

During the past summer my friend and colleague, Prof. E. C. Starks, dredged upwards of a hundred specimens of an agonoid fish, *Hypsagonus quadricornis*, in Puget Sound at a depth of approximately forty fathoms. The area over which the dredging extended was in the neighborhood of Friday Harbor and embraced an area of at least two hundred square miles where the bottom varied from sand to mud. Of the 37 specimens preserved in the Stanford University collection 10 of them are coated with a new species of hydroid, *Perigonimus pugetensis*, whose description is given later. In every specimen the cœlenterate was more abundant on the ventral surface of the body, especially in the axilla, and a luxuriant growth was usually found on the pectoral, ventral and, to a less extent, on the anal and caudal fins. With

[1] *Zool. Anz.*, Bd. 32, p. 752, 1908.

one or two exceptions the polyps were much more sparsely distributed over the body and dorsal fins. In no case were they found on the head.

Alcock, referring to several species of fishes of the family Scorpænidæ that "have the body and fins capriciously covered with long, wavy often tufted cutaneous filaments," believes, with a large company of zoologists, that these structures "assist in giving the fish a deceitful resemblance to the incrusted rocks of its environment, in order to allure, or at any rate not to scare, prey. And it appears probable that *Stylactis minoi* enables its companion *Minous inermis* in the same way to assume the same convenient and successful disguise." While the evidence is

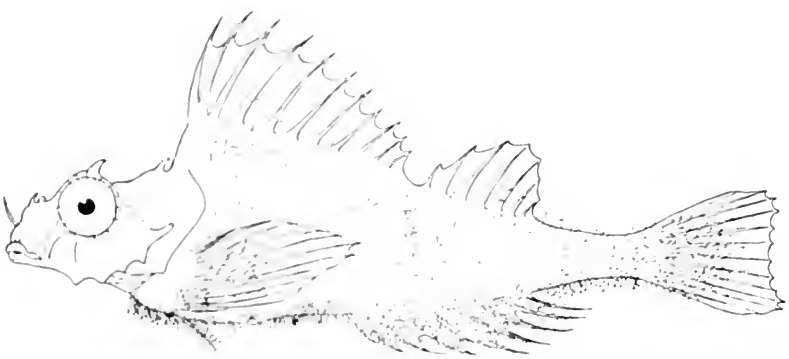

FIG. 1. An agonoid fish (*Hypsagonus quadricornis*) bearing a hydroid colony (*Perigonimus pugetensis*). Natural size.

strong that these devices do enable their possessor to escape detection and wage more successfully its battle for existence, and while the hydroid may enable the fish in question to more closely harmonize with its surroundings it does not follow even then that this is a case of commensalism. Nevertheless, as Hickson points out,[1] the fact that "the fish is never found without this hydroid, nor the hydroid without this species of fish, suggests very strongly that there is a mutual advantage in the association."

In the present case the evidence is not so cogent. About one fourth of the fishes only were overgrown with the hydroid and

[1] "Camb. Nat. Hist.," Vol. I., p. 268.

other specimens taken by the U. S. F. C. Str. "Albatross" in the open ocean off the Washington coast and in Bering Sea, are totally without them. These last named specimens, coming from the same depth (40 fm.) occurred on a pebbly bottom or one of broken shell and it is possible that the Puget Sound individuals, without the cœlenterate, occurred in a similar habitat. Be that as it may, it is a suggestive fact that in the fishes under consideration the hydroid was "attached in large numbers about the gill opening, on the throat and in the axilla," in other words over the ventral surface that is already the most concealed portion of the body. Referring to *Hypsagonus quadricornis* Prof. C. H. Gilbert writes in Jordan and Evermann's "Fishes of North and Middle America" (p. 204): "In the aquarium the fish appears to walk, resting alternately on the upper and lower pectoral rays and on the front rays of the anal." Under such circumstances the eddies produced in the bottom ooze would naturally bring the greatest amount of organic material to animals ventrally situated. The appearance strongly suggests that the advantage lies rather with the hydroid just as it does with the several species of barnacles attached to the skin of the whale. Whether the association is any more intimate in the case Alcock cites it is impossible to state conclusively, but the evidence is certainly not entirely convincing.[1]

Prof. C. C. Nutting, to whom I have submitted specimens, has kindly identified them as a species of *Perigonimus*, its nearest relative being apparently *P. vestitus* Allman. As in other members of the genus the hydrorhiza forms a highly branched, frequently anastomosing, system over the surface of the fish, but so far as noticed this contact is purely superficial, there being no evidence of parasitism. And furthermore the presence of small entomostracans and nondescript organic remains in the gastric

[1]Since this paper was sent to press I have examined upwards of two dozen specimens of this same species of rock perch (*M. inermis*) collected by my colleague Prof. J. O. Snyder, at Onomichi, on the Inland Sea, in the Province of Bingo, Japan. All of these are excellently preserved and in no instance has a hydroid been found upon them. It thus becomes more certain that the association described by Allcock is not an undoubted case of commensalism. Professor Snyder has called my attention to the fact that according to Regan (Ann. and Mag. Nat. Hist., 1905, Vol. XV., p. 20) *Minous inermis* should be *Minous monodactylus* (Bloch and Schneider).

cavity of the hydranths shows the feeding processes to be those of a non-parasitic species.

At frequent intervals branches, 3 to 4 mm. in height when fully developed, spring from this root system and each is terminated by a single hydranth. In no case does a hydranth arise as a lateral bud from the hydrocaulus, as in *P. vestitus*, for example. On the other hand, the medusa buds almost invariably appear as isolated, very rarely closely associated pairs of outgrowths dis-

FIG. 2. Portion of hydroid colony (*P. pugetensis*).

posed at comparatively regular intervals along the stem. Their order of appearance is seemingly not so definite, though this, in part at least, is perhaps due to the escape of an unknown number of medusæ from the older stems. On the shorter, younger branches one bud appears usually in the vicinity of the base or the hydranth, and about the time its development is half way completed a second one arises in the middle section of the stem,

while a third frequently makes its appearance in the vicinity of the hydrorhiza about the time the first medusa is liberated. Beyond this point the order of development is not known, but Fig. 2 illustrates a few of several different stages. The mode of development of the medusa is typical, and results in a bi-tentaculate type.

With the exception of the distal portion of each hydranth, including the tentacles, the entire colony is ensheathed in a cuticle often coated with minute organisms and sediment. In the older portions this investment is comparatively firm with the exception of that surrounding the hydranths, which is less dense and more flexible. The medusa buds are likewise covered, and for a time prior to their detachment are bound to the stem by an irregular cuticular bridge.

In the younger hydranths the line of demarcation between them and the stem is not clearly defined, but as they become older the boundary is more distinct, the hydranth growing more globular owing, to some extent at least, to the greater height of the endoderm. In the younger stages each hydranth bears four tentacles, later four others appear, often with slight irregularities in the time intervals, and finally with the appearance of four more the number is complete.

The following diagnosis will distinguish the present species from other known forms: *Perigonimus pugetensis* new species, twelve tentacles. Hydranths arising invariably from the hydrorhiza, and bearing as many as four scattered bi-tentaculate medusæ. Cuticle relatively thin. Occurs on the agonoid fish, *Hypsagonus quadricornis*, in Puget Sound, Washington.

THE CHROMOSOMES IN THE OÖGENESIS, FERTILIZATION AND CLEAVAGE OF COREID HEMIPTERA.

CHARLES V. MORRILL.

I. Introduction.

The recent conclusions regarding sex-production based on the nuclear dimorphism of the spermatozoa in the tracheates have involved certain assumptions regarding oögenesis and cleavage which, though made in conformity with many well-determined facts, are still in need of more adequate support from direct observations. The principal of these assumptions is that in the formation of the polar bodies, the diploid chromosome-groups of the female are reduced to haploid groups that are alike in all the mature eggs. Further, since the spermatozoa are of two sorts, the embryos produced would be correspondingly different and this difference should be apparent from a study of the embryonic nuclei. At present, however, the complete chromosome-cycle has been worked out in only two groups of tracheates, the phylloxerans and aphids. In the phylloxerans, Morgan ('08, '09) has traced the full history of the chromosomes through several generations and the combined observations of Stevens ('05*a*, '06*a*, 09) von Baehr ('08, '09) and Morgan ('09) practically completes the cycle in the aphids. The very recent observations of Boveri and Gulick ('09), though as yet published only in brief outline, show that in *Heterakis*, a nematode, the chromosome-cycle is similar to that assumed for many insects and bears the same relation to sex-production, while Baltzer ('08) has found that in sea-urchins, the conditions are the reverse of those in insects, the eggs being dimorphic and the spermatozoa all of one kind.

The present work[1] was undertaken with the hope of demonstrat-

[1]This problem was begun in the zoölogical laboratory of Columbia University and completed in the anatomical laboratory of the College of Medicine, Syracuse University. A part of the material was collected while occupying a John D. Jones Scholarship room at the biological laboratory, Cold Spring Harbor, N. Y., during the summer of 1907, and a Wistar Institute room at the Marine Biological Labor

ing the chromosome groups in the oögenesis, fertilization and cleavage in certain coreid hemiptera and of determining in this way, if possible, whether the assumptions made in regard to the number and behavior of the chromosomes in these stages is in accordance with the facts.

Four species of the Coreidæ were examined: *Archimerus alternatus* Say, *Anasa tristis* De Geer, *Protenor belfragei* Hagl., *Chelinidea vittigera* Uhler.[2] In all of these forms the spermatogonia have been found to contain an odd number of chromosomes, one of which (the unpaired idiochromosome[3]) fails to divide in one of the maturation divisions. One half the spermatozoa thus contain this chromosome, the other half lack it, and a dimorphism of the spermatozoa arises. The oögonia have been shown to have an even number of chromosomes, the unpaired element of the spermatogonia being represented here by a pair of equal size. The maturation of the egg had not been fully worked out, but it was assumed that every chromosome divides equally in both polar divisions, giving to the mature egg a group of chromosomes similar to that borne by a spermatozoön having the idiochromosome. The eggs were accordingly assumed to be all of one kind with respect to their chromatin content, as direct observation has shown to be true in phylloxerans (Morgan), aphids (Stevens, von Baehr) and more recently in *Heterakis* (Boveri and Gulick.)

atory, Woods Hole, Mass., in 1909. To the directors of these laboratories I am indebted for the facilities placed at my disposal. I wish also to express my gratitude to Professors E. B. Wilson and T. H. Morgan for the many helpful suggestions they have made during the progress of this work.

[2] I am indebted to Mr. E. P. Van Duzee, of Buffalo, N. Y., for the identification of my material. The species *Archimerus alternatus* is almost, if not quite, identical with the *A. calcarator* of Professor Wilson's material (identified by Mr. P. R. Uhler) and was collected from the same locality in Van Cortlandt Park, New York. *Protenor* was found at Woods Hole, Mass., and *Anasa* at Woods Hole, and Cold Spring Harbor, N. Y. *Chelinidea* was taken by Professor Wilson at Southern Pines, N. C. A part of the living specimens of *Anasa* were also furnished by Professor Wilson.

[3] For the sake of simplicity, the term "idiochromosomes" will be used in this paper to designate those chromosomes which are associated with sex-production, irrespective of their detailed behavior in the growth periods or maturation divisions. It will thus include the "idiochromosomes" (in the more restricted sense), "accessory," "odd" or "heterotropic" chromosomes, "monosomes," "heterochromosomes," "X- and Y-elements," etc., of recent writers. Professor Wilson has used this term in a similar sense in the fourth of his "Studies" ('09*b*).

It was further supposed that if an egg is fertilized by a spermatozoön bearing the idiochromosome an embryo will result whose nuclei all have an even number of chromosomes, similar to the oögonial groups, but if fertilized by a spermatozoön lacking that chromosome, the embryonic nuclei will have an odd number of chromosomes similar to the spermatogonial groups. Accordingly the former will be females, the latter males. It should be possible, then, to distinguish the sex of an embryo by an inspection of the embryonic (somatic) chromosome groups. Again, if the number of chromosomes in the male and female pronuclei could be accurately determined just before the first cleavage spindle is formed, it would afford additional evidence of the dimorphism of the spermatozoa and the relation this condition bears to sex-production.

II. Material and Methods.

The insects were brought into the laboratory or greenhouse and placed in cages in which their food plants were growing. Here they paired readily and laid their eggs either on the plants or on the sides or bottom of the cages. The breeding periods of the four genera employed differ widely. *Anasa* may be found pairing on squash plants in the vicinity of New York or Woods Hole early in July, the eggs being laid in clusters on the under surfaces of the leaves, but specimens kept in a greenhouse over the winter laid early in May. *Chelinidea*, brought from the south and kept in a greenhouse during the winter, began to lay its clusters of eggs in the latter part of March. *Archimerus* begins laying on the goldenrod in the vicinity of New York in the latter part of May or first of June. The eggs are laid singly, and it was found impossible to collect sufficient numbers in the field so that all those used were taken from caged individuals. *Protenor* also lays its eggs one at a time and, in the laboratory, rarely makes any attempt at fastening them to any object, but drops them to the bottom of the cage where they were collected in small quantities. In addition to these, a number of eggs were taken from the oviducts of *Anasa* and *Protenor*.

The eggs of the four species differ markedly in size, *Archimerus* having the largest and *Protenor* the smallest, those of *Anasa* and

Chelinidea being intermediate; these size differences correspond roughly with the difference in size of the several species. All the eggs, whether in the oviduct or after laying, are enclosed in a tough brown chorion.

Several different fixing fluids were tried. Flemming's strong fluid, Gilson's mercuro-nitric and Bouin's picroformol were found very uncertain in result, as they seldom penetrate the thick chorion. All these can be used, however, if the eggs are pricked with fine needles before placing them in the fixing fluid, but their action is such as to render the yolk very brittle and difficult to cut. By far the best results were obtained by placing the eggs immediately in the Gilson-Carnoy acetic-alcohol-chloroform-sublimate mixture for fifteen to thirty minutes or in a mixture of glacial acetic, one part, absolute alcohol saturated with sublimate, two parts, for five to ten minutes. After either fluid, the eggs were transferred to iodized 95 per cent. alcohol for twelve hours and preserved in 80 per cent. alcohol. The acetic-alcohol-sublimate mixture was found invaluable for the earliest stages of maturation which occur while the eggs are still in the lower part of the oviduct and directly after laying. For later stages, the Gilson-Carnoy mixture gave excellent results. After immersion in alcohol, the egg shrinks away from the chorion which can then be removed with fine forceps and cutting needle. After removing the chorion, the eggs were dehydrated, cleared in cedar oil and immersed in melted paraffin for two hours. They were then oriented in a drop of paraffin and embedded. Serial sections were cut 6-8 μ thick on a sliding microtome. Very good series can be obtained in this way though the yolk sometimes becomes brittle and troublesome. The stain most frequently employed was iron-hæmatoxylin with or without a counter-stain. In addition to the eggs, ovaries and testes were fixed in Flemming's strong fluid and stained in iron-hæmatoxylin or safranin.

About twelve hundred eggs in all were sectioned, but owing to mechanical difficulties in technique, only about two hundred of these were of any value for study. In the maturation and fertilization stages particularly, one or two poor sections may render an entire series worthless, though in later embryonic stages this difficulty is not so serious. For this reason the results are

necessarily somewhat meagre, but they are perfectly clear as far as they go.

III. Descriptive.

A. *Oögenesis.*

The results on oögenesis are confined to the chromosomes of the oögonial and oöcyte divisions. No attempt has been made to trace the full history of the growth period, but an examination of a few eggs taken from the ovarian tubes seems to show that no definite chromatin-nucleolus or persistent oögonial chromatin element is present, as stated by Wilson ('06). The nucleus at this time contains many faintly staining chromatin threads and several small nucleoli whose nature was not determined. Foot and Strobell ('09) have found the same condition to be true in *Euschistus*, a pentatomid.[4]

1. *Archimerus alternatus.*

The spermatogonial groups[5] have been figured by Wilson in the second of his "Studies" ('05*b*), but for the sake of comparison two more are shown (Fig. 1, *c* and *d*). Each group has 15 chromosomes, two of which, the *m*-chromosomes (following Wilson's terminology), can always be identified by their very small size. Of the remaining thirteen no one can be positively identified, by its size or shape, as the idiochromosome. In the spermatocyte divisions this chromosome passes undivided to one pole of the spindle in the *first* mitosis and divides equally in the second (Wilson, '05*b*). This condition is peculiar to *Archimerus* alone of all the Coreidæ so far described,[6] but a rëexamination of the spermatocyte stages in new material shows beyond doubt that Wilson's account is correct. The idiochromosome can be easily identified in the first mitosis by its peripheral position on the spindle and by its unconstricted contour when the other chromosomes are in early, and even late, anaphase. It passes to one pole of the spindle a little behind the others. Further, in the

[4]Stevens ('06*b*) has described "heterochromosomes" in certain stages of the growth period of the oöcytes of *Aphrophora*, an homopteran.

[5]See footnote 2, at bottom of page 80.

[6]Professor Wilson has also found a similar condition in *Pachylis gigas* (unpublished).

cysts of second spermatocytes, one finds metaphase groups with eight and seven chromosomes side by side.

The oögonial groups[7] have not been figured previously, but Wilson gives the number of chromosomes as 16 in the fourth of his "Studies" (09*b*). In Fig. 1, *a* and *b*, two plates are shown

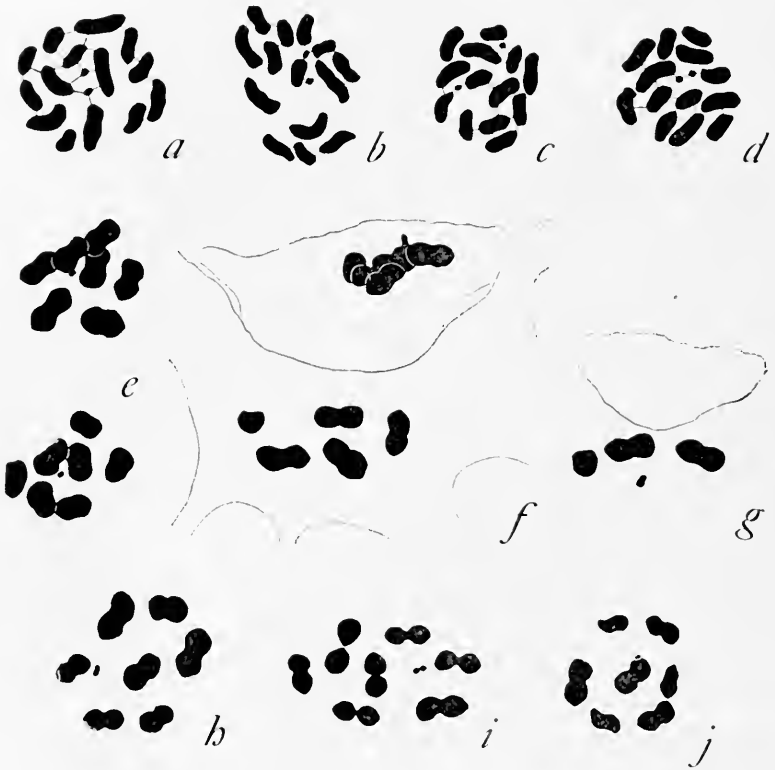

FIG. 1. *Archimerus alternatus*. *a* and *b*, oögonial, *c* and *d*, spermatogonial metaphase-groups. *e–j*, first oöcyte-(polar) division; *e*, daughter groups from the same anaphase-spindle, polar view; *f* and *g*, anaphase, side view, in two sections; *h* and *i*, inner daughter groups, polar view, of two anaphases; *j*, outer daughter group, polar view, of an anaphase.

each with 16 chromosomes. A pair of *m*-chromosomes again appears in each group. The fourteen larger elements present a somewhat graded series and cannot be paired off readily. Two

[7]The unreduced female groups in all the species, were taken from the region in the ovary lying just below the nutritive chamber, where oögonia, follicle-cells and young oöcytes are found together. For convenience they will be called "oögonia."

of the largest are probably the idiochromosomes, though they do not differ sufficiently from the others, in size or shape, to admit of exact identification.

The anaphase of the first mitosis was the earliest oöcyte stage obtained and was found in eggs just after laying. Fig. 1, *e* (from two sections), shows a polar view of this stage in which eight chromosomes can be accurately counted in both outer and inner groups. There are seven large dyads and one very small one in each, corresponding in relative size to the fourteen large and two small chromosomes of the oögonia. Fig. 1, *f* and *g*, show a side view of a late anaphase from two sections. The inner group contains eight dyads; one of them has divided prematurely, the two parts appearing in neighboring sections. The chromosomes in the outer group are too crowded to be counted. Pl. I., *a*, shows another anaphase in which the inner group is complete in one section and contains eight dyads. Fig. 1, *h* and *i*, show the inner groups of two more anaphases, polar view. The chromosomes in both are all dyads, eight in each, the component parts of which show all degrees of separation. This premature division of the dyads is very common in the final anaphases of the first division and might lead to mistakes in counting if it were not that one finds all stages of division up to the complete separation shown in Fig. 1, *f* and *g*. Henking found this condition in the egg of *Pyrrhocoris* ('92, Pl. III.) and it has also been found in the first spermatocytes of *Aphrophora* an homopteran (Stevens, '06*b*) and *Anax* a dragon-fly (Lefevre and McGill, '08). It is probable that even in cases of extreme separation, the halves of the dyads remained connected by fine strands of chromatin or linin which become invisible after long extraction of the stain. The second division is thus foreshadowed, in the anaphase of the first, and even before as will be shown in *Anasa*. Since there is no period between the first and second divisions when the chromosomes lose their individual contour, in fact no telophase in the strict sense, the dyads pass practically unchanged into the second maturation spindle.

The chromosomes which enter the first polar body retain their contour and grouping for some time, forming a flat plate when seen in surface view. Fig. 1, *j*, shows such a plate with seven

large chromosomes all more or less constricted and a small nodule close to the central one, which probably is the *m*-chromosome. Fig. 2, *b*, shows another in somewhat oblique view with seven large dyads, and the *m*-chromosome dyad, the latter faintly stained. Fig. 1, *f*, and Pl. I., *a*, show side views of two more polar body groups; in Fig. 1, *f*, the *m*-chromosome dyad is dis-

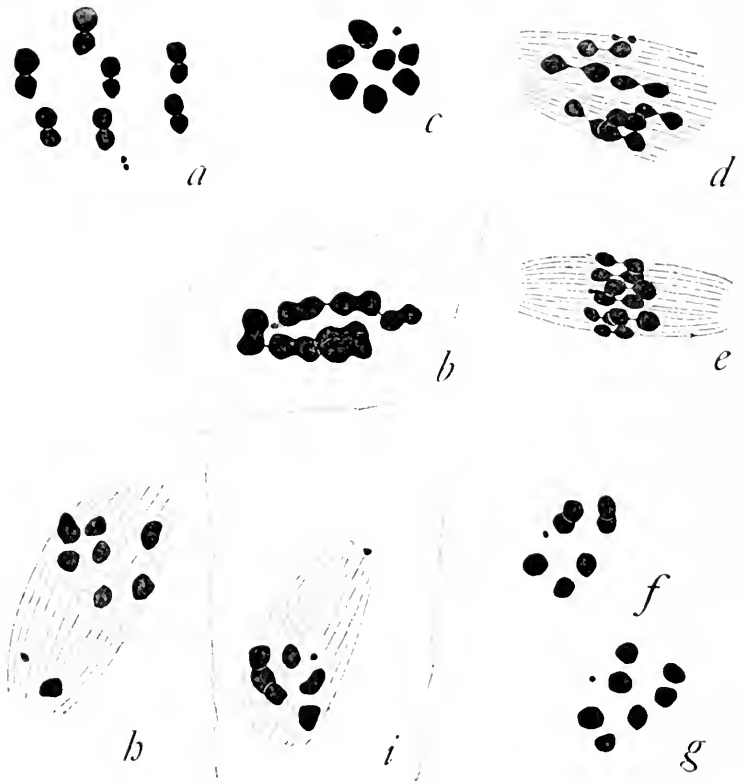

FIG. 2. *Archimerus alternatus*. Second oöcyte-(polar) division. *a*, metaphase-group, oblique view. *b*, chromosome-group of the first polar body from the same egg as the last. *c*, metaphase-group, polar view. *d* and *e*, metaphase groups, side view. *f* and *g*, daughter groups from the same anaphase-spindle, oblique view. *h* and *i*, anaphase, oblique view, in two sections.

tinctly seen. All the chromosomes show the typical constriction as though ready for a second division. However no spindle is formed and no division takes place except that in individual chromosomes the halves of a dyad may separate of their own accord,

just as in those of the inner groups. At the close of the second division the chromosomes of the first polar body are finally merged together in a deeply-staining mass.

The second division follows closely upon the first as stated above. The chromosomes do not crowd together in the final anaphase of the first division as in the spermatocytes, but merely rotate about forty-five degrees and become disposed upon a new spindle which forms out of the enveloping cytoplasm. Fig. 2, *a*, shows a metaphase of the second division in slightly oblique polar view. The seven large dyads and *m*-chromosome dyad are sharply constricted and ready for division. The first polar body with its eight dyads taken from the same section is shown in Fig. 2, *b*. Fig. 2, *c*, shows another polar view of a second division metaphase, and Fig. 2, *d* and *e*, are side views of the same, each showing eight chromosomes. All the dyads are more or less drawn out in preparation for division, and in anaphase, separate into two groups of monads. Fig. 2, *f* and *g*, show outer and inner groups respectively taken from the same anaphase; each has eight monads. Fig. 2, *h* and *i*, show an oblique view of an anaphase in two sections (the surface of the egg is indicated by a dotted line at the right of each section). The outer group which passes into the second polar body is shown above, the seven large monads in *h*, the *m*-chromosome monad in *i*. The inner group which remains in the egg, is shown below; six of the large monads and the *m*-chromosome monad in *i*, the remaining large monad in *h* (The stippled object in *h* is part of one of the chromosomes in *i*). Thus the end result is, that the female pronucleus is formed from eight single elements or monads, comparable to those borne by a spermatozoön containing the idiochromosome (vid. Wilson, '06).

There are no peculiar or "lagging" chromosomes in either division. Which of the polar divisions is reducing, *i. e.*, separates whole chromosomes which have paired in synapsis, could not be determined, since the first oöcyte prophases were not obtained.

While it is not possible to identify the idiochromosomes in the two divisions just described, the *m*-chromosome appears as a constant element dividing in both. In the spermatocytes Wil-

son's ('05*b*) figures show it occupying the centre of spindle in both divisions. However, in the oöcytes its position is variable in the first division (Fig. 1, *e*, *h*, and *i*; Pl. I., *a*) but always peripheral in the second (Fig. 2, *a*, *c*, *d*, *f* and *g*, *h* and *i*).

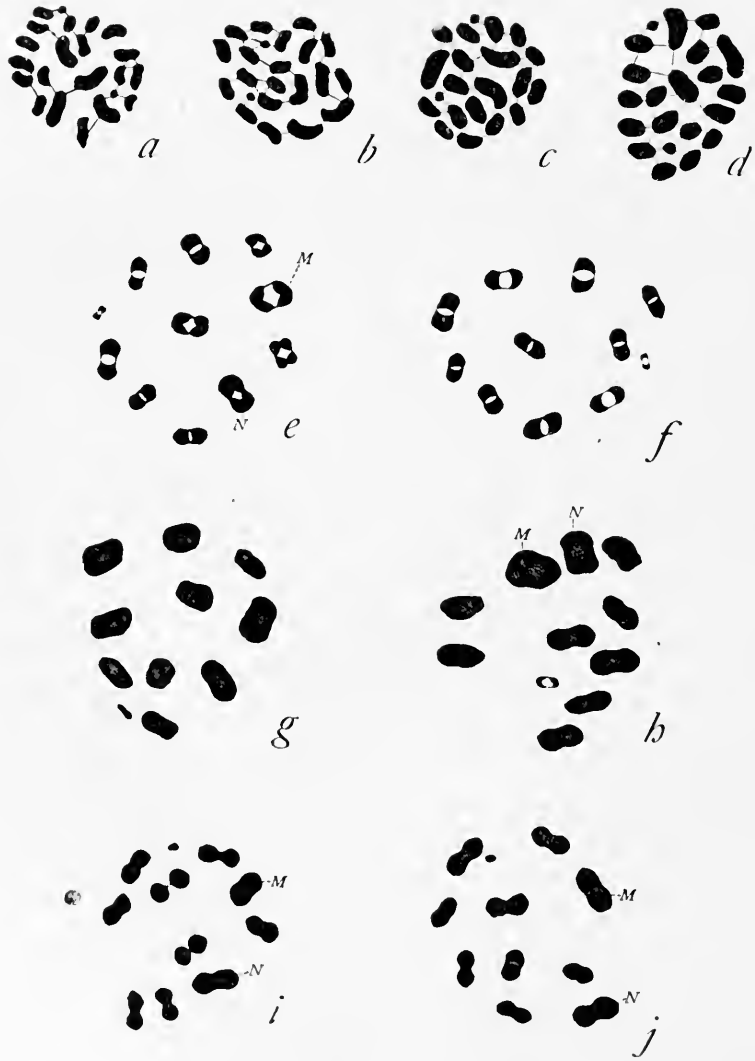

FIG. 3. *Anasa tristis*. *a* and *b*, oögonial, *c* and *d*, spermatogonial metaphase-groups. *e*–*j*, first oöcyte-(polar) division: *e*, *f*, *g* and *h*, metaphase-groups, polar view. *i* and *j*, daughter groups from the same anaphase-spindle, polar view.

2. *Anasa tristis.*

The spermatogonial groups have been figured by Wilson ('05*b* and '06), and the number of chromosomes stated as 21. Wilson's count has been corroborated by Montgomery ('06) and by Lefevre and McGill ('08) though Paulmier ('99) and Montgomery in an earlier paper ('01) had given the number as 22. Foot and Strobell ('07*a* and 07*b*) have disputed the twenty-one count and, on the basis of photographs of smear preparations, have concluded that the number is 22, confirming Paulmier's original account. A reëxamination of fresh material from two different localities gave 21 as the correct number of spermatogonial chromosomes in agreement with accounts of Wilson, Montgomery, and of Lefevre and McGill. Imperfect plates it is true may show less than this number, but none were found with more. In *Chelinidea vittigera*, where the spermatogonial groups are almost identical with those of *Anasa*, a single exception may be cited, in which 22 chromosomes were found. Apart from this, all clear plates gave 21.

As pointed out by Wilson and by Lefevre and McGill, the spermatogonial groups of *Anasa* show two *m*-chromosomes and three largest chromosomes one of which must be the unpaired chromosome ("heterotropic" or "accessory" chromosome) though it cannot be precisely identified by its size or shape. Fig. 3, *c* and *d*, shows two spermatogonial groups illustrating these points.

The oögonial groups (Fig. 3, *a* and *b*) contain 22 chromosomes, including two *m*-chromosomes and four largest chromosomes. Of the last named, two must be the idiochromosome pair, corresponding to the unpaired idiochromosome of the spermatogonia, as pointed out by Wilson in the third of his "Studies" ('06).

The metaphase of the first oöcyte division was found in eggs taken from the lower part of the oviduct. Fig. 3, *e*, *f*, *g* and *h*, shows four such phases, *h* being a composite figure from two sections.* There are 11 chromosomes in each, all undoubtedly

*The difference in size between the chromosomes as a whole of Fig. 3, *e* and *f*, and those of Fig. 3, *g* and *h*, is probably due to a difference in the length of fixation. In the former case the eggs were left for ten minutes in the fixative (alcohol-acetic-sublimate), in the latter, for five minutes. The chromosomes of Fig. 3, *g* and *h*, seem to show the swelling action of the acetic unchecked by the sublimate which penetrates more slowly.

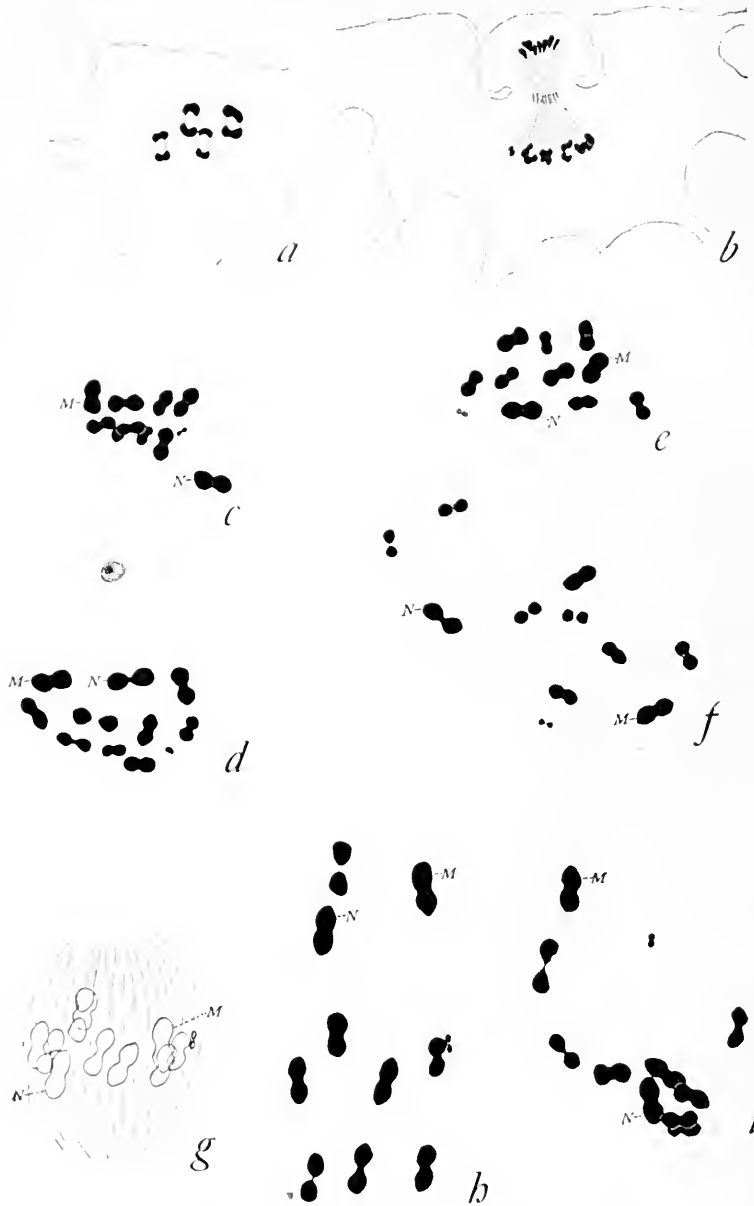

FIG. 4. *Anasa tristis*. *a–f*, first oöcyte-(polar) division; *a*, four chromosomes in initial anaphase, side view, showing tetrad-character; *b*, final anaphase, side view; *c* and *d*, daughter groups from the same anaphase-spindle, slightly oblique polar view; *e* and *f*, the same from another egg; *g*, metaphase-group, side view, second oöcyte-(polar) division. *h*, the chromosomes of the last drawn at three different focal levels. *i*, the chromosome-group of the first polar body from the same eggs

double, arranged in an irregular ring with one in the centre and one or two outside. Unlike the first spermatocytes, the central chromosome is not the *m*-chromosome bivalent but one of the larger ones, while the *m*-chromosome itself lies just within the ring (Fig. 3, *e*, *f* and *h*) or somewhat outside (Fig. 3, *g*). In these four metaphases, the axis of the spindle is somewhat oblique to the plane of section and the structure of the chromosomes can thus be readily observed. As might be expected many have the shape of typical tetrads foreshadowing the two oöcyte divisions. This is especially well seen in Fig. 3, *e*, where even the *m*-chromosome has the quadripartite form. Whether distinctly quadripartite, or simply dumb-bell shaped, the plane of the first division is clearly indicated in every chromosome. In Fig. 3, *e* and *h*, there are two bivalents (*M* and *N*) perceptibly larger than any of the others. In all probability these arise from a pairing of the four largest chromosomes of the oögonia. Accordingly one of them may be considered to be the idiochromosome bivalent.

The anaphase of the first polar spindle was found in eggs directly after laying.[9] The chromosomes divide in the plane already indicated at metaphase. Fig. 4, *a*, shows a side view of four chromosomes in initial anaphase; the tetrad character of each is clearly indicated. Fig. 3, *i* and *j*, shows polar views of the outer and inner groups respectively from the same spindle; each has 11 chromosomes. All, with the exception of the *m*-chromosome in *j*, are obviously dyads showing all degrees of constriction, as in the first polar anaphase of *Archimerus*. The outer group (*i*) which passes into the first polar body shows two dyads completely divided though the polar body itself neither divides nor forms a spindle. In both groups two largest dyads (*M* and *N*) can be distinguished. Fig. 4, *c* and *d*, are outer and inner groups respectively from another spindle, in slightly oblique polar view (in *c* the large chromosome, *N*, was found in the same section as the inner group, *d*). Both groups contain 11 dyads of which two (*M* and *N*) are larger than any of the others. Fig. 4, *e* and *f*, are outer and inner groups respectively of a third anaphase in

[9] A single exception to this was found. One egg taken from the lowest part of the oviduct showed the first polar spindle in initial anaphase It is possible that rough handling started the maturation process, as has been found in other groups of animals.

somewhat oblique polar view (the entire outer group, *e*, and six chromosomes of the inner group, *f*, appeared in one section, the remaining five of the latter group, in the next section). Both groups contain 11 dyads of which several in *f* show premature separation of their parts.

The peripheral position assumed by the *m*-chromosomes in metaphase (Fig. 3, *e*, *f* and *g*) is again seen in all the anaphase groups (Fig. 3, *i* and *j*; Fig. 4, *c*, *d*, *e* and *f*). This is undoubtedly their normal position in the first polar division. A side view of the final anaphase (Fig. 4, *b*) shows no peculiar or "lagging" chromosome on the spindle. All the chromosomes divide equally, as the polar views of three final anaphases show, where every chromosome is distinctly visible.

A single example of the second polar division was found (Fig. 4 *g*). It is a side view of a metaphase with 11 dyads. In *h*, the dyads of this group are drawn at three different focal levels. The first polar body (*i*) of the same egg also contains eleven dyads. In both the second polar spindle and the first polar body, there are two dyads (*M* and *N*) somewhat larger than any of the others. These are undoubtedly the products of division of the two largest tetrads of the first polar metaphase (Fig. 3, *e* and *h*). The *m*-chromosome in the second division (Fig. 4, *g* and *h*) takes a peripheral position as it did in the first. It appears also in the first polar body (*i*). In short there are three chromosomes distinguishable by their size, which can be identified in both polar divisions. These in all probability arise from a pairing of the four largest and two smallest oögonial chromosomes.

Though no anaphases of the second division were found, it is almost certain from the results in *Archimerus* that all the dyads divide equally, in a plane corresponding to the constriction shown at metaphase. Accordingly the female pronucleus, as well as the second polar body, will contain 11 monads and correspond to a spermatozoön bearing the idiochromosome as assumed by Wilson ('06).

3. *Protenor belfragei*.

The spermatogonial groups have been described and figured by Montgomery ('01 and '06) and by Wilson ('06). These observers agree that there are 13 chromosomes, one of which is

more than twice as large as the next in size. Of the remaining twelve, two are much larger than the others, and the rest form a graded series of pairs. The smallest pair, the *m*-chromosome, is relatively larger in this species than in *Archimerus* and *Anasa*. In Fig. 5, *c* and *d*, are shown two spermatogonial groups illus-

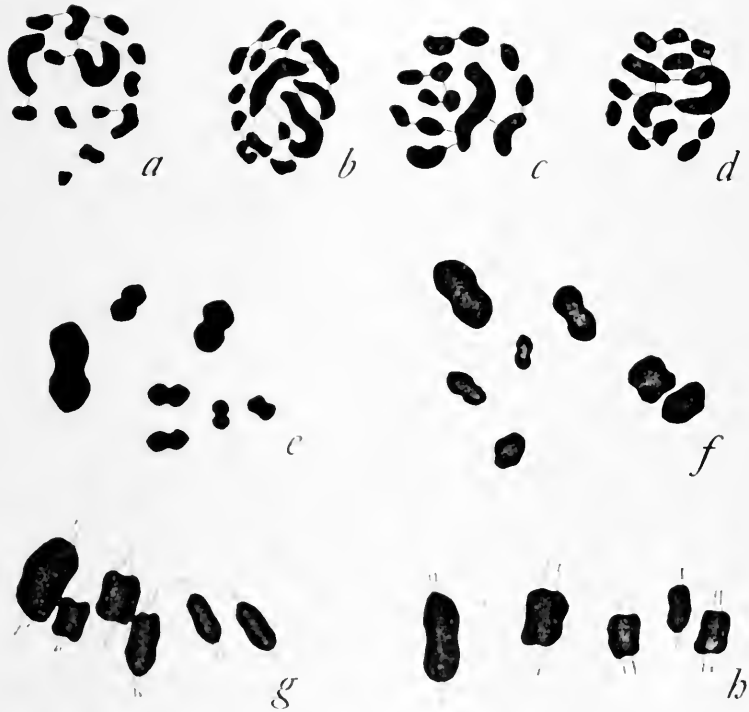

FIG. 5. *Protenor belfragei*. *a* and *b*, oögonial. *c* and *d*, spermatogonial metaphase-groups. *e–h*, first oocyte-(polar) division; *e*, metaphase group, polar view; *f*, metaphase group, oblique polar view; *g*, six chromosomes in metaphase, side view; *h*, five chromosomes in metaphase, side view.

trating the above mentioned points. The idiochromosome does not appear constricted as figured by Montgomery ('01), Fig. 134.

The oögonial groups were figured by Wilson in the third of his "Studies" ('06). In them "there are two very large chromosomes, equal in size, in place of the single one that appears in the male, while the remaining chromosomes show the same relations as in the male." In Fig. 5, *a* and *b*, two of these groups are shown.

Of the maturation stages, only four preparations were obtained,

all of the first polar metaphase (Fig. 5, *e*, *f*, *g* and *h*).[10] Two of these (*e* and *f*) are complete, showing seven bivalents. The idiochromosome bivalent can be positively identified by its relatively enormous size, having been formed, no doubt, by the synapsis of the two largest chromosomes (idiochromosomes) of the oögonia. It does not in any way resemble a nucleolus. The bivalent next in size, corresponding to the two next largest chromosomes of the oögonia, can also be identified. Indeed the bivalents as a whole show the same relative size differences as the chromosome pairs in the oögonia. The *m*-chromosome bivalent is the smallest but only slightly smaller than the next in size. In Fig. 5, *f*, three chromosomes of intermediate size are seen in face view; two of these exhibit a quadripartite form, clearly indicating their bivalent nature. Fig. 5, *g*, is a side view of an incomplete metaphase, drawn from two sections, showing six of the seven chromosomes, and Fig. 5, *h*, shows a metaphase, side view with only five chromosomes, again taken from two sections. In both of these figures the idiochromosome bivalent can be readily identified by its size and the plane of the second division is clearly indicated in all the bivalents. While the further processes of maturation were not followed, it may be inferred, on the analogy of *Archimerus*, that in the anaphase of the first division the seven tetrads divide into two groups of dyads and the inner group of these separate in the second division into two groups of monads; the inner group of these last named, seven in number, enter into the formation of the female pronucleus, which is thus similar in chromatin-content to a spermatozoön bearing the idiochromosome.

4. *Conclusions Regarding Oögenesis.*

The results on maturation are somewhat meagre it is true but perfectly clear as far as they go, and point to the conclusion that, unlike the spermatozoa, all the mature eggs are of one kind with respect to their chromatin-content, as has been assumed. The female pronucleus contains a reduced group of chromosomes similar in size and number to that carried by a "female-pro-

[10] The excessive size of the chromosomes in these figures, especially those of *g* and *h*, is probably due to the peculiar action of the fixative (see footnote 8, on page 89).

ducing" spermatozoön, *i. e.*, one bearing the idiochromosome (directly proved in *Archimerus*, but only in part in *Anasa* and *Protenor*). The idiochromosome bivalent is not distinguishable by its behavior from other chromosomes, and divides in both mitoses, giving an equal portion to each oötid. It never assumes a nucleolus-like form either in the oögonial or oöcyte divisions.

B. *Some Details of Polar Body Formation.*

The place of polar body formation has been found to vary in different groups of insects. It may be on the dorsal surface, either approximately midway between the poles as in *Blatta* (Blochmann, Wheeler) and *Pyrrhocoris* (Henking), or a short distance behind the anterior end as in *Musca* (Blochmann, Henking). In Chrysomelidæ (Hegner) it is ventral, while in *Pieris* (Henking) it is close to the anterior (micropylar) end. In *Hydrophilus* (Heider) and *Aphis* (Stevens) it is lateral. In *Archimerus* the polar bodies are given off on one of the flat surfaces of the egg, approximately midway between the poles. In this species the two surfaces cannot be distinguished after the chorion has been removed, but in *Protenor* the dorsal surface is markedly convex while the ventral is flat or slightly concave, and it was here determined by proper orientation that the place of polar body formation is on the dorsal surface. The first polar spindle lies in a small thickening of cytoplasm with its axis at right angles to the egg surface (Fig. 1, *f* and *g*; Fig. 4, *a* and *b*; Pl. I., *a*). The anaphase of the first polar division occurs just after laying (in *Archimerus* a single exception was found; see footnote 9, page 91). No centrosomes or asters could be demonstrated by the method of fixation used just as Henking ('92) found in *Pyrrhocoris* though he used a different method. In late anaphase a cell-plate is formed by swelling of the spindle fibers and the surface of the egg dips down and around the outer group of chromosomes, until finally a little mass of cytoplasm containing the latter comes to lie free in a depression of the egg surface (Fig. 1, *f* and *g*; Fig. 4, *b*; Pl. I., *a*). In both the first and the second polar divisions (Fig. 4, *b*, and Pl. I., *b*) the constriction probably does not involve the cell-plate but passes between it and the outer group of chromosomes as Henking observed in *Pyrrhocoris*.

In *Anasa*, a rounded cytoplasmic body was found, in two cases, in or near the first polar spindle (Fig. 3, *i*; Fig. 4, *c-d*). This is perhaps comparable to the "eigenthümliches Körperchen" which Henking described in *Pyrrhocoris*. It may be a plasmosome, but it is difficult to decide, since one frequently finds a number of cytoplasmic bodies in the neighborhood of the first polar spindle (Pl. I., *a*) which cannot be distinguished from yolk granules and which are inconstant in appearance and number.

At the conclusion of the first polar division the spindle gradually fades away; there is no persistent cylindrical mass of spindle fibers or "thelyid" as Henking ('90 and '92) found in *Pieris*, a lepidopteran, and *Agelastica*, a coleopteran. The chromosomes left in the egg, as stated before, remain separate and there is no telophase in the strict sense. After a short resting period, they rotate about 45° and become disposed on a new spindle which has formed out of the cytoplasm surrounding them. The axis of the second polar spindle lies very obliquely to the surface of the egg (Fig. 2, *h* and *i*; Pl. I., *b*). As in the first division there are no centrosomes or asters. In late anaphase a cell-plate is formed by swellings of the spindle fibers. The second polar body is constricted off in the same manner as the first and lies alongside of it in the same depression. The first does not divide. The two bodies finally become embedded in the surface cytoplasm and can be distinguished as late as the third or fourth cleavage.

At the close of the second polar division, the chromosomes left in the egg become massed together and are converted into the female pronucleus (Pl. I., *b*). Those which have entered the polar bodies may remain separate for some time but eventually fuse into one or two deeply staining masses.

C. *Fertilization.*

The spermatozoa enter the egg through the micropyles which form a conspicuous ring at the anterior end. Polyspermy is undoubtedly normal, for accessory sperm nuclei were found in the egg as late as the copulation stage shown in Pl. II., *b*. As many as three of these nuclei appeared in some cases. At the time when the first polar spindle is in late anaphase, the sperm head enveloped in a mass of cytoplasm has moved some distance

into the egg among the yolk spheres leaving a train of cytoplasm behind it. It appears as a compact deeply staining rod surrounded by a clear area and preceded by an aster (Pl. I., *d*). The clear area is probably the "arrhenoid" mentioned by Henking ('92) in his account of *Pyrrhocoris*. The sperm head often appears coiled at the end which points away from the direction of its movement. Later it loses its staining power and opens out into an oval vesicle (Pl. II., *a*). The clear area and aster are here well marked though no centrosome is visible in the preparation. Still later, the vesicle becomes considerably larger and small irregular masses of chromatin can be seen in its interior. It is then ready for copulation.

In the meantime, the egg nucleus, formed from the inner group of chromosomes of the second polar spindle, has begun to move into the egg surrounded by a small mass of cytoplasm (Pl. I., *c*). The cytoplasm frequently contains one or more yolk spheres. The nucleus is at first round in outline and the chromatin is distributed in small nodules lying chiefly against the nuclear membrane. Subsequently it loses its capacity for staining, and appears somewhat like the sperm head, but more rounded. It then begins to increase in size, becoming at the same time irregular in shape and the chromatin once more appears in irregular masses.

As the two pronuclei approach each other their cytoplasmic areas fuse and they come to lie side by side with an amphiaster between (Pl. II., *b*; the aster on the upper side of the nuclei was drawn from the next section). In contact with each pronucleus, may often be seen a large clear vesicle. These probably represent the structures mentioned by Henking as the "descendants of the arrhenoids," *i. e.*, derived from the clear area surrounding the male pronucleus. In Pl. II., *b*, one of the asters contains a minute centrosome. The entire amphiaster is probably formed under the influence of the male pronucleus, for, in the same egg from which Pl. II., *b*, was taken, an accessory sperm nucleus was found with a very small amphiaster lying in contact with it. The further history of the clear vesicles could not be followed as very few first cleavage figures were found; at a later stage of copulation (Pl. II., *c*) they did not appear. Henking

described them in *Pyrrhocoris* as forming the poles of the first cleavage spindle ("Polkörperchen") and apparently considered them to be archoplasmic masses. That he did not see an aster in front of the male pronucleus nor an amphiaster at copulation, in addition to these structures, is perhaps due to his methods of technique.

During the approach of the pronuclei the chromatin in each becomes more and more condensed until the compact somewhat elongated chromosomes appear. Pl. II., *c*, the single example of this stage found, shows the pronuclei of *Archimerus*, still slightly separated. An indistinct aster appears at the right. In the lower nucleus seven chromosomes of different sizes can be distinctly seen. The *m*-chromosome is missing and because of its small size could not be identified in the next section. There are no nucleoli in either pronucleus.[11] The chromosomes in the upper nucleus are not yet fully condensed. The two pronuclei are so nearly equal in volume that one cannot distinguish which is male and which female even before copulation (Pl. II., *c*). It is apparent from a comparison of Pl. II., *b* and *c*, that both undergo a marked decrease in volume just before their nuclear membranes fade out. Pl. II., *d*, shows a late copulation stage or prophase of the first cleavage spindle of *Protenor* in polar view. The nuclear membranes have faded out but the chromosome groups derived from each pronucleus are still separate. This figure is obviously incomplete but it shows distinctly one reduced group (at the right of the figure) in which all the chromosomes appear, seven in number. Just as in the first oöcyte division, the idiochromosome is here recognizable by its relatively large size, and does not in any way resemble a nucleolus. A next largest chromosome and an *m*-chromosome also can be identified, the remaining four being intermediate in size. The group at the left of the figure shows the idiochromosome and three others, the remaining chromosomes being too crowded in the next section to identify. Since each group contains an idiochromosome it is not possible to say which was derived from the egg nucleus and which from a sperm of the class which bear this chromosome.

[11]Stevens ('06*a*, Pl. IV., Fig. 119) has figured this stage in the "Goumi aphid" where there are five chromosomes in each pronucleus, and, in the female, two plasmosomes in addition.

However, the embryo arising from this union would have been a female, for all the products of the first cleavage nucleus would contain two idiochromosomes as in the oögonia. A chromosome group taken from a female embryo is shown in Fig. 12, *c*. Even these meagre results make it probable that the chromosomes coming out of the male and female pronuclei at copulation are of the same number and show the same relative size differences as those which previously entered into the formation of the gametic nuclei.

D. *The Cleavage and Blastoderm Nuclei.*

The cleavage nuclei are formed by successive division of the fertilization nucleus. After each division the daughter nuclei move apart, each surrounded by a star-shaped cytoplasmic island. They wander toward the periphery, continually dividing by mitosis and there form the blastoderm. No instances of amitosis were observed in these stages such as Wheeler ('89) described in *Blatta*. Although but few first cleavage divisions were found, the chromosomes in them do not differ from those of somewhat later stages described beyond. The cleavage mitoses all show spindles, centrosomes and asters with diagrammatic clearness and the chromosomes, though somewhat elongated, can be counted as readily as in the oögonial or spermatogonial divisions. In metaphase each chromosome appears on the spindle split lengthwise and in anaphase the halves separate as in ordinary homotypic division. In telophase the chromosomes at either pole become vesicular, fuse together and form a daughter nucleus. At first the contents of the resting nucleus entirely lacks staining power, no nucleoli of any kind appearing. As the time for the subsequent division approaches, small flakes of chromatin appear which increase in number and gradually unite to form the chromosomes. In the cleavage stages, no definite chromatin nucleoli or plasmosomes could be seen with the methods of fixation employed, nor was there any elimination of chromatin during the earlier mitoses as described by Boveri in *Ascaris*.

1. *Archimerus alternatus.*

A careful study of the eggs after fertilization revealed the fact that there are two sorts of embryos, one having 15 chromosomes

in all its cleavage—and blastoderm—nuclei and the other 16. These chromosome numbers are the same as those found in the spermatogonia and oögonia respectively. The size-relations also

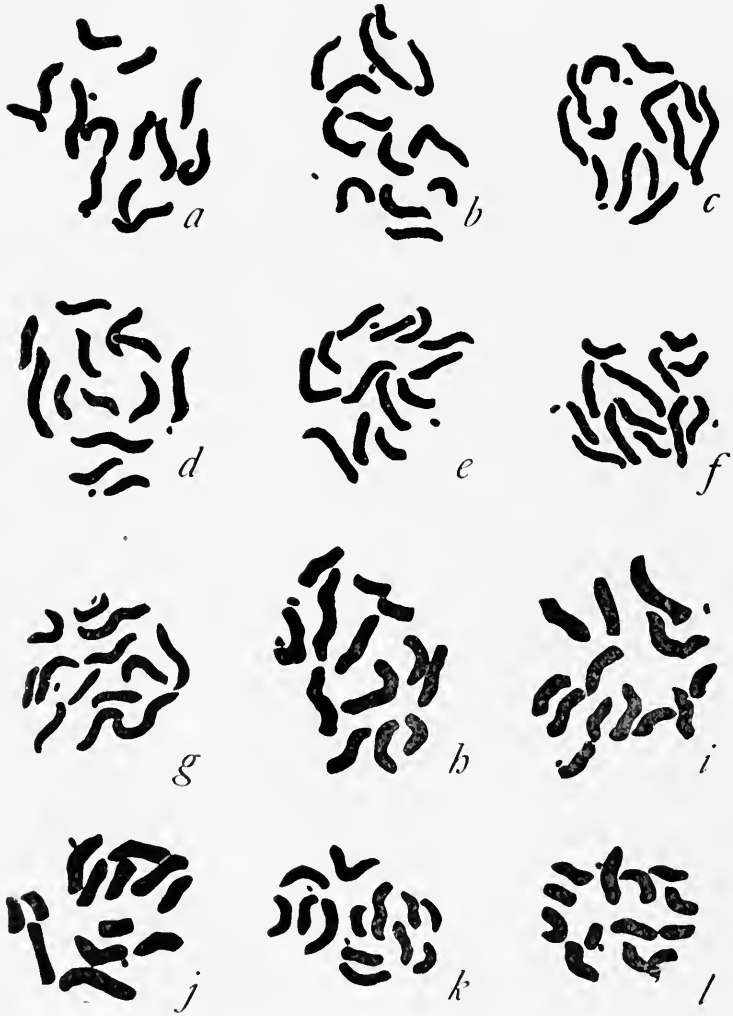

FIG. 6. *Archimerus alternatus*. Chromosome-groups of embryonic cells, 15-chromosome type. *a–e*, from the same embryo; *f–l*, from other embryos.

are in general the same as in the gonads. It seems fair then to conclude that the 15-chromosome embryos are males, the 16-chromosome, females. In Fig. 6 are shown twelve 15-chromo-

some groups taken from embryos at different stages. Fig. 6, *a*, *b*, *c*, *d* and *e*, are from the same embryo early in the formation of the blastoderm. Fig. 6, *f* and *g*, are from another embryo in the same stage. Fig. 6, *h* and *i*, are from an embryo in a slightly later stage of the blastoderm. Fig. 6, *j*, is from still another at the same stage as the last, and *k* from a late blastoderm. Fig. 6,

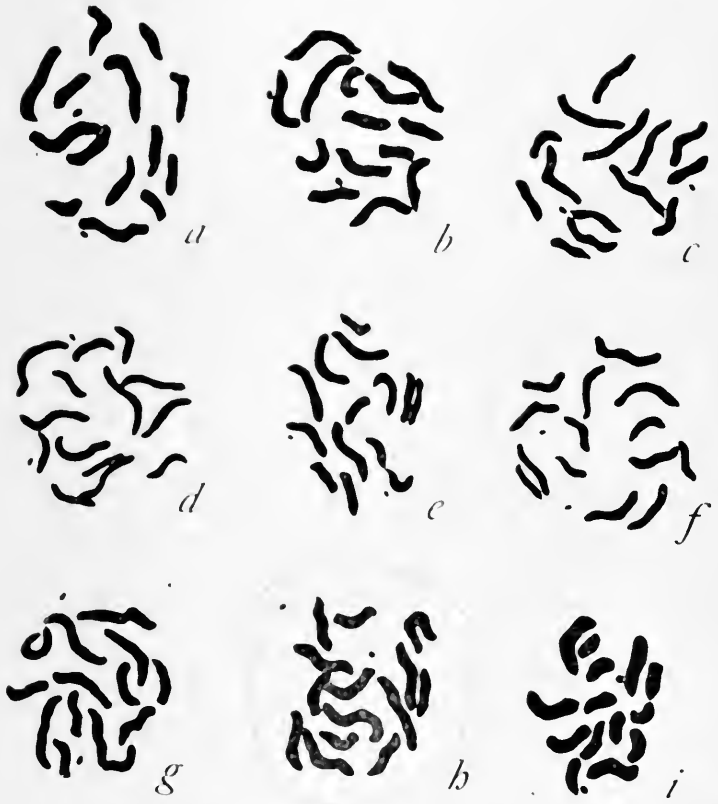

FIG. 7. *Archimerus alternatus*. Chromosome-groups of embryonic cells, 16-chromosome type. *d*, *e*, and *f*, from the same embryo; others from different embryos.

l is taken from a stage in which the blastoderm is invaginating to form the embryonic fundament and membranes. The 16-chromosome groups are shown in Fig. 7. Fig. 7, *a*, shows a group taken from the interior of an egg in the cleavage stage. Fig. 7, *b* and *c*, are from an embryo in the early blastoderm stage.

Fig. 7, *d*, *e* and *f*, are from another embryo in the same stage as the last. Fig. 7, *g* and *h*, are from a slightly later blastoderm and *i* is from a completed blastoderm.

An inspection of Figs. 6 and 7 as a whole, shows that in the earlier stages, the chromosomes are somewhat elongate (Fig. 6, *a–g*; Fig. 7, *a–f*) and that as development proceeds, they become shorter and thicker until at the time of invagination or just before (Fig. 6, *k–l*; Fig. 7, *i*) they have about the same contour as those of the spermatogonia and oögonia (compare with Fig. 1, *a–d*). In every stage the *m*-chromosomes, though very minute, are constant elements. The unpaired idiochrosome in the male groups and the paired idiochromosomes in the female cannot be distinguished by their size or contour but are probably represented among the larger chromosomes. The remaining chromosomes cannot be readily paired off.

2. *Anasa tristis.*

The embryonic mitoses of *Anasa*, though having a larger number of chromosomes that those of *Archimerus*, are much more favorable for making chromosome counts, especially in the early (incomplete) blastodern stage, *i. e.*, at a time when many of the cleavage nuclei have reached the surface and are still rapidly dividing. The embryos are of two classes: one having 21 and the other 22 chromosomes. Since these numbers correspond to those in the spermatogonia and oögonia respectively, it may be concluded that the 21-chromosome class are males, the 22-chromosome class, females. Fig. 8, *a–h*, show eight metaphase groups from the 21-chromosome class. Fig. 8, *a*, *b*, *c*, *d*, *e* and *f*, are taken from an embryo in the early blastoderm stage. In this embryo ten more perfectly clear groups were found each with 21 chromosomes, making sixteen in all from the same embryo. Fig. 8, *g* and *h*, are from another embryo in the same stage. In Fig. 9 six groups of the 22-chromosome class are shown, all from the same embryo in the early blastoderm stage.

One exceptional group was found in an embryo of the 22-chromosome class (Fig. 8, *i*). This group contains 23 chromosomes, of which three are larger than the rest. It is difficult to suggest an explanation for this condition. It may be due to an

accident of technique, the microtome knife tearing a chromosome in half as it passed through the block, or it may be the result of an abnormality in a previous division. There were no other

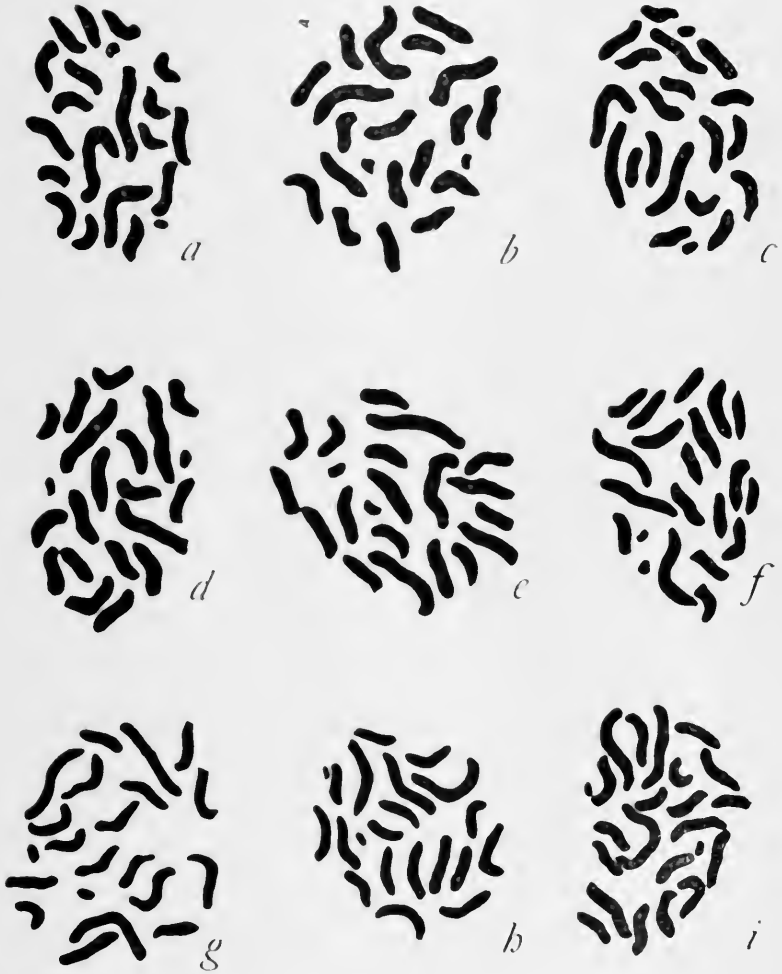

FIG. 8. *Anasa tristis*. Chromosome-groups of embryonic cells. *a–h*, 21-chromosome type: *a*, *b*, *c*, *d*, *e* and *f*, from the same embryo; *g* and *h*, from another embryo. *i*, exceptional group with twenty-three chromosomes.

mitoses in the immediate vicinity from which an extra chromosome could have been derived. The chromosome groups represented in Figs. 8 and 9 were selected from a large number of

very clear preparations. Many more could have been shown, but it seemed needless to multiply the number of figures.[12]

A comparison of the male groups (Fig. 8, *a–h*) with the female groups (Fig. 9) shows clearly that in the former there are three chromosomes larger than the rest, while in the latter there are four such elements. These size relations are the same as those in the spermatogonia and oögonia respectively (vid. page 89).

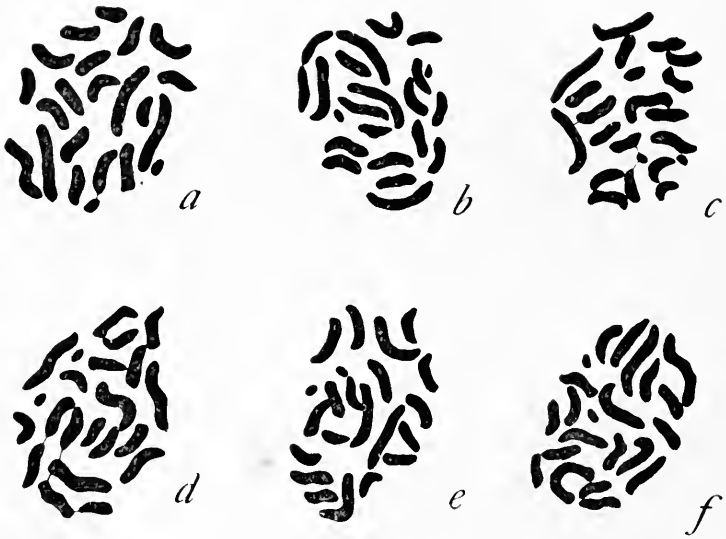

FIG. 9. *Anasa tristis*. Chromosome-groups of embryonic cells, 22-chromosome type, all from the same embryo.

Accordingly, one of the large chromosomes of the male groups must be the unpaired idiochromosome and two of the large chromosomes of the female groups, the paired idiochromosomes. The *m*-chromosomes appear as constant elements in both groups and are usually more elongated than in the germ-cells, a feature which is common to all the chromosomes. Apart from the largest and smallest elements, this elongated condition makes it impossible to pair off the remaining chromosomes with any degree of certainty.

[12]All the figures were drawn with camera lucida, Zeiss apochromat. 2 mm., compens. oc. 12. With the exception of Plates I. and II., they were again enlarged with the camera and subsequently reduced in reproduction one-half, giving a final magnification of 2,650 diameters. The magnification of Plates I. and II., is 1,375 diameters. Achromatic structures, except those of Plates I. and II., have been represented semi-schematically.

3. *Chelinidea vittigera.*

The embryonic groups of this species are very similar to those of *Anasa* and equally favorable for making chromosome counts. The embryos are again of two sorts, one having 21 chromosomes, the other 22. In fact the number and size-relations are so much like those in *Anasa* that the two forms cannot be distinguished by their chromosome complexes alone.

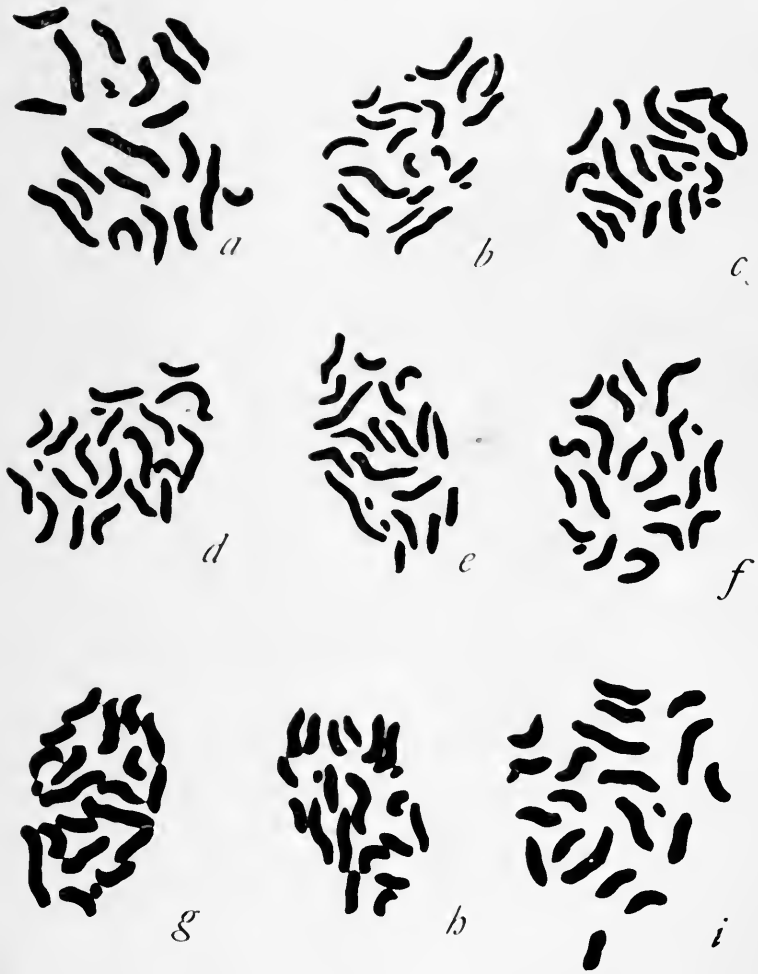

FIG. 10. *Chelinidea vittigera.* Chromosome-groups of embryonic cells. *a–h*, 21-chromosome type; *b*, *c*, *d*, *e* and *f*, from the same embryo; *a*, from an early cleavage. *i* exceptional group with twenty-two chromosomes.

In Fig. 10, *a–h*, are shown eight 21-chromosome groups. Fig. 10, *a*, is from an early cleavage stage corresponding approximately to the fourth cleavage of holoblastic eggs.[13] Fig. 10, *b*, *c*, *d*, *e*, and *f* are from an early blastoderm stage. Seven more perfectly clear counts were made in this embryo, all giving 21 chromosomes, making twelve in all. Fig. 10, *g* and *h*, are from another embryo in the same stage. Fig. 11 shows six of the 22-chromosome groups. Fig. 11, *a*, is taken from an early cleavage

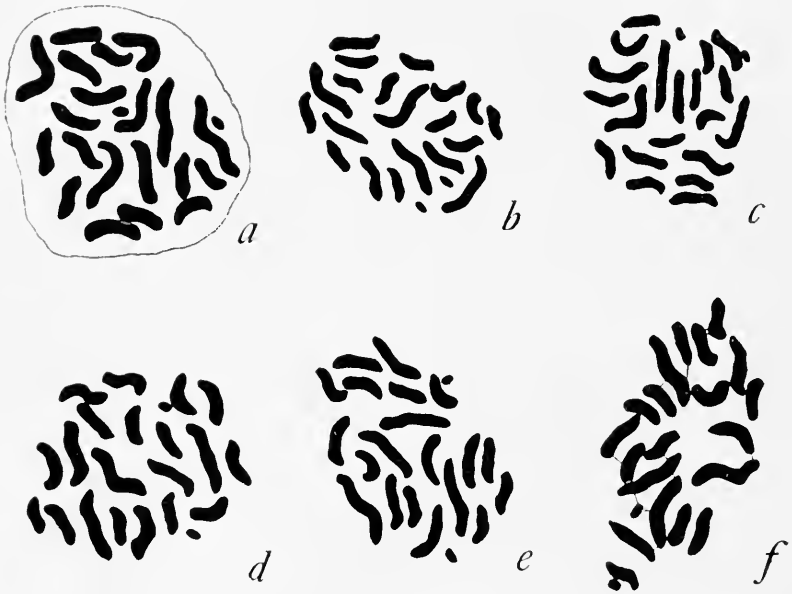

FIG. 11. *Chelinidea vittigera*. Chromosome-groups of embryonic cells, 22-chromosome type. *b*, *c*, *d* and *e*, from the same embryo; *f*, from another embryo; *a*, from an early cleavage.

stage, approximately the fourth. Fig. 11, *b*, *c*, *d* and *e*, are from an early blastoderm stage in which eleven perfectly clear groups were found all with 22 chromosomes. Fig. 11, *f*, is from another early blastoderm.

As in *Anasa*, it seems fair to conclude that the embryos with 21 chromosomes are males, those with 22 chromosomes, females,

[13]After the "second cleavage," the nuclei apparently do not divide quite synchronously so that one may find at times an odd number of nuclei, some resting and some in division.

for these groups correspond in number and size-relations with those of the spermatogonia and oögonia respectively. Like *Anasa* again, the male groups contain three chromosomes which are distinctly larger than the others (Fig. 10, *a–f* and *h*), one of them probably representing the unpaired idiochromosome. In the female groups four largest chromosomes can frequently be distinguished (Fig. 11, *b*, *c* and *f*), though they cannot be identified in all the figures, probably on account of fore-shortening. Two of these may be considered as the paired idiochromosomes, in place of the unpaired element of the male groups. The *m*-chromosomes are typically paired elements of both male and female groups. All the chromosomes as in *Anasa* are more elongated than in the gonads but still preserve the same size-relations. The groups shown in Figs. 10 and 11 were selected from a large number of clear preparations. Fig. 10, *i*, is a group of 22 chromosomes taken from an embryo in which all other counts gave 21. For this, the single exception of its kind observed, it is difficult to give a satisfactory explanation, though the same possibilities suggested in connection with the exception found in *Anasa* (vid. page 102) might also apply here. There were no neighboring groups from which the extra chromosome could have been derived.

4. *Protenor belfragei.*

The embryonic mitoses of this species are not quite so favorable for making chromosome counts as those of the two preceding forms, on account of the elongation of the chromosomes in the early and even late cleavage stages. In the blastoderm stage the chromosomes are more compact, but very few embryos were obtained at this time so that the results are very meagre. Fig. 12 shows two 13-chromosome groups (*a* and *b*) taken from an early blastoderm stage, and one 14-chromosome group (*c*) from another embryo in the same stage.

In the 13-chromosome groups (Fig. 12, *a* and *b*) a very large chromosome, unquestionably the unpaired idiochromosome, stands out clearly, being more than twice as large as any other. A second largest pair can also be readily identified and a smallest pair, the *m*-chromosomes.

In the 14-chromosome group (Fig. 12, *c*) there are two very large chromosomes equal in size, in place of the unpaired element of the first two groups. These are no doubt the paired idiochromosomes. There is also a next largest pair but the *m*-chromosomes cannot be identified with certainty.

A comparison of these embryonic mitoses (Fig. 12) with those of the gonads (Fig. 5, *a–d*) shows that the 13-chromosome groups are similar in the number and size-relations of their chromosomes

FIG. 12. *Protenor belfragei*. Chromosome-groups of embryonic cells. *a* and *b*, 13-chromosome type, from the same embryo. *c*, 14-chromosome type, from another embryo.

to those of the spermatogonia, the 14-chromosome group, to those of the oögonia. Though the results are too few to justify broad conclusions, it is most probable that the embryo with 13 chromosomes is a male, the one with 14 chromosomes a female, thus bringing *Protenor* in line with *Archimerus*, *Anasa* and *Chelinidea*.

IV. Summary and Conclusion.

Among the results described in this paper,[14] those of particular interest are as follows:

1. In *Archimerus*, *Anasa* and *Protenor* there is an odd or unpaired chromosome in the spermatogonia which in *Protenor* is distinguishable by its size. The oögonia contain in addition to this chromosome, a second chromosome of the same size. These observations are in agreement with those of Wilson, Montgomery and of Lefevre and McGill for the forms mentioned.

2. The chromosomes in the reduced female groups (polar or oöcyte divisions) show the same relative size differences as the corresponding pairs in the oögonia (particularly well shown in *Protenor*).

3. All the chromosomes divide in both polar divisions (proof

[14] A preliminary note giving the most important results was published in *Science* for December 31, 1909.

decisive in *Archimerus*, less complete in *Anasa* and *Protenor*). There are no peculiar or "lagging" chromosomes in either of these divisions.

4. The female pronucleus contains a group of chromosomes similar to that borne by a spermatozoön having the "accessory" or idiochromosome (directly proved in *Archimerus*).

5. At fertilization the reduced groups from each pronucleus are separately distinguishable and the chromosomes show the same size relations as those of the spermatocyte and oöcyte divisions. There are no nucleoli in either pronucleus.

6. In the cleavage and early blastoderm nuclei of *Archimerus*, *Anasa*, *Chelinidea* and *Protenor*, the chromosomes are perfectly distinct and can be counted as readily as those in the gonads. Two types of embryos are found, one having an odd and the other an even number of chromosomes, these numbers being respectively the same as occur in the spermatogonia and oögonia. Accordingly it seems fair to conclude that the former are males, the latter females, and it thus becomes possible to distinguish the sex of an embryo by counting its chromosomes.

7. The idiochromosomes behave exactly like the other chromosomes, in the oöcyte divisions, at fertilization and in the cleavage and early blastoderm stages. They never show any resemblance to nucleoli and in *Protenor* they can be identified in all stages with absolute certainty.

It will be seen that the results in general bear out the assumptions made by Stevens, Wilson and others regarding the number and behavior of the chromosomes in the maturation of the female and in the somatic cells. They give additional morphological support to theories of sex-production based upon the presence or absence of certain chromosomes and to the hypothesis of chromosome-individuality or "genetic continuity of chromosomes" as Wilson ('09c) more cautiously calls it.

V. Review and Discussion.

The literature on the maturation and early development of the eggs of insects and allied forms is very extensive, covering a period of over fifty years, but it is beyond the scope of the present paper to review it in detail except in so far as it concerns

the history of the chromatin in the early stages. Considered from this standpoint, the results briefly are as follows:

Diptera.—Apart from the earlier works of Weismann and Blochmann in which the chromosomes were not especially considered, there are no observations except those of Henking ('88 and '93) on *Musca vomitoria*. In the first paper Henking figured the cleavage spindles but did not determine the number of chromosomes. Moreover, the results are difficult to interpret because of the standpoint taken in regard to "free-nuclei-formation." In the later paper Henking summarizes his previous results but gives no new observations.

Lepidoptera.—Platner ('88) described briefly the maturation and early cleavage of the parthenogenetic and fertilized eggs of *Liparis dispar* but gave no figures and no account of the chromosomes. Henking ('90) described and figured the maturation, fertilization and early cleavage of *Pieris brassicæ*. He found the haploid number of chromosomes to be 14 in both polar spindles and in the female pronucleus, but did not accurately determine the diploid number, though in a later paper he gives the probable number as 28. The same author ('92) gave a brief account of the maturation and early cleavage of *Bombyx mori* and *Leucoma salicis* in which the haploid chromosome-group was stated to be "at least 12," in both species. The diploid number was not determined.

Neuroptera.—As far as I am aware, there are no observations on the maturation and cleavage of mitoses of the eggs of this group. Miss McGill's ('06) observations on *Anax junius* and *Plathemis lydia* were confined to the nuclear changes during the growth period of the oöcytes.

Coleoptera.—Wheeler ('89) observed the formation of the first polar spindle of *Leptinotarsa* (*Doryphora*) *decemlineata* but did not determine the number of chromosomes. Henking ('92) in *Agelastica alni* found the haploid number of chromosomes to be about 12 in both polar spindles and the diploid number, 24–30 in the cleavage spindles. He also observed the approximate number in *Lampyris splendidula*, *Adimonia tanaceti*, and *Donacia* (*sericea* L.?). In none of these, however, did he observe the diploid groups in the cleavage stages. The observations of

Giardina ('01) on *Dytiscus marginalis* and likewise those of Debaisieux ('09) on the same species were confined to the growth period of the oöcytes. Both authors describe a large chromatic mass in the nucleus of the oöcyte, distinct from the chromosomes, which appears to be eliminated just before the maturation divisions. The latter are not described.

Orthoptera.—The observations of Blochmann ('87) and Wheeler ('89) on *Blatta germanica* were not very extensive from our point of view. Neither author determined the number of chromosomes in the maturation spindles. Wheeler, however, gives a good figure of a cleavage spindle showing 10 chromosomes. Gutherz ('07) in a brief paper, described a chromosome-nucleolus in the oöcytes of *Pyrrhocoris* but found no such body in the somatic mitoses of *Gryllus domesticus*. He therefore questioned the occurrence of "heterochromosomes," maintaining that there were probably 20 chromosomes in the somatic cells of the last named species and no "heterochromosomes." However in his later papers ('08 and '09*a*) he abandoned this view, describing typical "heterochromosomes" in the spermatogonia and oögonia of *Gryllus* as in other Orthoptera, and stating further, that the somatic cells have the same number of chromosomes as the oögonia and spermatogonia respectively though he gave no observations in support of this last statement. The observations of von Baehr ('07) on the parthenogenetic egg of the phasmid, *Bacillus rossii*, though detailed in some respects, are not quite conclusive in regard to the number of chromosomes. The egg nucleus, just before the first polar division, contains 18–20 chromosomes many of which are tetrads. In anaphase the double nature of the daughter halves often becomes apparent. Moreover, there is one large tetrad in the first division which again appears in the second. The number of chromosomes in the latter division was not determined. In a recent paper Buchner ('09) has described in *Gryllus campestris* an irregular nucleolus-like structure, the "accessory body," which persists through the growth period of the oöcytes. It is derived from a similar body in the oögonia which he apparently considers identical with the "accessory" chromosome of other forms. The maturation mitoses were not observed. Gutherz ('09*b*) working on a nearly related species, *Gryllus domes-*

ticus, finds a similar body in the growth period of the oöcytes. After tracing its history, he concludes however that it is comparable to a nucleolus and is not to be confused with a "heterochromosome." Buchner's identification of the "accessory body" as a chromosome thus appears very doubtful.

Hymenoptera.—Blochmann's ('89) observations on the maturation of the fertilized and parthenogenetic egg of the bee were concerned chiefly with the number of polar bodies formed in the two sorts of eggs. Though the polar spindles were figured the exact number of chromosomes was not determined. Henking ('92) found the haploid number of chromosomes to be 10 in the polar spindles of *Lasius niger* and the diploid number in the cleavage spindles, 20. In the unfertilized egg of *Rhodites rosæ* he found 9 chromosomes in the polar spindles. In the cleavage nuclei the number was 18–20, *i. e.*, the number of chromosomes in the female pronucleus had been doubled. Petrunkewitsch ('01) working on the fertilized and parthenogenetic eggs of the bee, found that the first polar division was equational, the number of chromosomes being 16. In the second division there occurred in both sorts of eggs, a reduction of the chromosome-number to about half, *i. e.*, from 16 to 8. In the parthenogenetic (drone-) egg, the female pronucleus contained at first 8, but later 16 chromosomes, the latter being produced by a doubling of the haploid group, so that in the equatorial plate of the first cleavage spindle the diploid number, 16, again appeared. In later cleavages there was a progressive doubling of the chromosome-number, producing multiple groups of 32 and 64. Silvestri ('06 and '08) has described in detail the maturation, fertilization and cleavage of several species of parasitic hymenoptera (*Litomastix*, *Encyrtus*, *Oöphthora*, *Ageniaspis*). However, since his results do not include the determination of the exact number of chromosomes in the early stages, it will be unnecessary to review them here. Doncaster's ('07) results on *Nematus ribesii* (Tenthredinidæ) are quite anomalous and difficult to interpret. He finds that there are two types of maturation in the female. In some eggs there is no reduction of chromosomes, the female pronucleus receiving the diploid number, 8. In others typical reduction occurs, the egg nucleus receiving in all probability the haploid number, 4.

The former type of egg develops parthenogenetically, the latter only being capable of fertilization, it is supposed. In some somatic tissues, such as the ovary sheath, there are more than the diploid number of chromosomes, as in the bee and in *Ascaris*. In a later brief communication, however, Doncaster ('10*a*) states that his observations on the polar mitoses may require revision and that the behavior of the chromosomes in *Nematus ribesii* is so difficult to follow that it is doubtful if a satisfactory interpretation can be obtained in this species. In a very recent paper, the same author ('10*b*) describes in detail the maturation, fertilization and early cleavage of *Neuroterus lenticularis* (Cynipidæ). Here again some of the results are quite novel. The mitoses of the primitive ova found in young female larvæ of the summer generation contain about 20 chromosomes, like those of the somatic cells. In the maturation of the summer eggs apparently two divisions occur, the female pronucleus probably containing 10 chromosomes. The eggs are fertilized and in the cleavage spindles about 20 chromosomes appear. The results on the maturation of the spring (parthenogenetic) egg are so anomalous that it seems best to quote from Doncaster's own summary (*loc. cit.*, p. 102): "The maturation of the spring egg has not yet been sufficiently studied, but it appears that some eggs undergo at least one maturation division, others probably none. In eggs in which maturation has occurred segmentation mitoses show 10 chromosomes; all the eggs laid by one individual female in which the chromosomes could be counted were of this type, and it is suggested that these develop into males. In the eggs laid by other females, however, 20 chromosomes appear in the segmentation divisions; in these, polar chromosomes appear to be absent, and it is probable that there has been no maturation division, and that these eggs would develop into females." It will be seen that no definite conclusions can be drawn without further confirmatory observations. Schleip's ('08) observations on the polar body formation in *Formica sanguinea* were confined chiefly to the parthenogenetic egg. He found in the latter the haploid number of chromosomes to be about 24 in the maturation spindles and female pronucleus. This number also appears in the first cleavage nucleus. In the fertilized egg, the number of chromosomes

in the male and female pronuclei was not determined with certainty but is probably 24 in each. On the whole, the chromosomes were small and the size-differences not well marked.

Hemiptera-homoptera.—The older papers of Weismann, Witlaczil, Blochmann and Will on the early embryology of aphids contain no detailed account of the chromosomes. Stschelkanovzew ('04) in a brief paper on the maturation and early cleavage of the summer (parthenogenetic) egg of *Aphis rosæ*, gave 14 as the number of chromosomes in the maturation spindle (only one polar body is formed). In one first cleavage spindle there were only 11 chromosomes but he considered that three of these might be double elements, thus giving the diploid number, 14, in both maturation and cleavage. Miss Stevens ('05*a*) and Hewitt ('06),[15] however, found the diploid number in the parthenogenetic egg of *Aphis rosæ* to be 10 and this count has been confirmed by von Baehr ('09) in the same species. Miss Stevens also found that the winter (fertilized) egg gave off two polar bodies in which the haploid number, 5, was present. Three of these authors observed marked size differences in the chromosomes of the maturation and early cleavage stages, both Miss Stevens and von Baehr finding four smallest chromosomes constantly. Miss Stevens ('06*b*) in a very extensive paper described the maturation and cleavage in a large number of aphids, with especial reference to the number and behavior of the chromosomes. Without giving a detailed review of her results it may be said in general that the number and size differences of the chromosomes was found to be constant for the species and that this constancy applies to the diploid groups whether in the maturation spindle of a parthenogenetic egg or in its cleavage spindles. In many cases also the haploid group was found to exhibit the same relative size differences as the diploid group of the same species. In the fertilized egg of the "Goumi aphid," the number of chromosomes in the male and female pronuclei just before copulation was shown to be 5 in each, *i. e.*, the haploid number. Miss Stevens concluded from her observations on aphids up to this time that there were no "heterochromosomes" in this group, but

[15]Hewitt's results are known to me only through the brief mention made by von Baehr ('09, p. 285).

more recently ('09) she has abandoned this view and has reached conclusions in agreement with those of Morgan and von Baehr, mentioned below. The results of Tannreuther ('07) on the maturation and cleavage of several species of aphids differ in many important respects from those of other workers on the same group. They have been questioned by Morgan ('09) and von Baehr ('09) and will not be considered here. Morgan ('08 and '09) has traced the full history of the chromosomes through several generations of phylloxerans. He observed that the parthenogenetic eggs of the second generation, *i. e.*, those which produce the sexual individuals, are of two sorts both as to their size and the number of chromosomes in the embryos which they produce. The male embryos have actually two less chromosomes than the female though this difference is not always apparent owing to fusions occurring between certain chromosomes (the "accessories.") The embryonic chromosome-groups of female individuals contain four "accessory" chromosomes. Those of the males, however, have but two "accessories" since although males are produced parthenogenetically from females, two of these chromosomes are given off to the polar body in the maturation of the male-producing egg. The most important conclusions to be drawn from these results for our purposes are, that idiochromosomes are present throughout the entire life-cycle and that it is possible to diagnose the sex of an embryo by counting its chromosomes, though here it is true, sex is also associated with the size of the egg. Von Baehr ('09) described the maturation and cleavage of the parthenogenetic eggs of several species of aphids. His results were in general similar to those of Miss Stevens, the maturation and cleavage mitoses being similar in the number and size relations of the chromosomes. He did not observe any elimination of "accessory" chromosomes in the polar division of male eggs as in the phylloxerans. However, in one maturation spindle of *Aphis saliceti* (*loc. cit.*, Pl. XIV., Fig. 42) he figures 5 chromosomes, the remaining figures showing 6, and in a male somatic cell (Pl. XV., Fig. 94), as well as in the spermatogonia, 5 chromosomes again appear. Moreover his results on the spermatogenesis of *Aphis saliceti* as well as those of Miss Stevens ('09) on the spermatogenesis of other aphids would seem

to indicate that the behavior of the chromosomes in the female line is probably similar to that in phylloxerans.

Hemiptera-heteroptera.—The only observations on the maturation and cleavage of the egg in this group are those of Henking ('92) on *Pyrrhocoris*. He has given a very extensive and detailed account of the chromosome history in this form and in a previous paper ('91) described the spermatogenesis. He found that in the diploid groups of the oögonia, there were 24 chromosomes. The follicle and connective tissue cells, both larval and adult also showed this number. In the haploid group of the first polar spindle 12 dumbbell-shaped chromosomes appeared. In one such group ('92, Pl. III., Fig. 83) one chromosome is much larger than the rest and is probably the idiochromosome pair (cf. Wilson's ('09*d*) figures of the oögonial groups). The second polar spindle showed again 12 dumbbell-shaped chromosomes. The number of chromosomes in the male and female pronuclei was not accurately determined but in Henking's Fig. 90 (Pl. III.) one such nucleus shows 12 chromosomes. The early cleavage spindles were figured but of them the author says (*loc. cit.*, pp. 29–30): "The number (of chromosomes) cannot be accurately determined on account of the smallness of the spindle and the close grouping of the chromosomes . . . it should be 24." He thus did not distinguish two classes of embryos with reference to the chromosome number. This, no doubt, was partly due to the fact that he had not observed any difference in the number of chromosomes in the spermatogonia and oögonia and did not appreciate the significance of the idiochromosome ("accessory" chromosome) which he himself was the first to describe. Foot and Strobell ('09) have described the growth period of the oöcytes of *Euschistus variolarius*. In accordance with the earlier account of Wilson ('06), they find no chromatin nucleolus in the young oöcytes or germinal vesicles of this species but in the older oöcytes and in the germinal vesicles there is a relatively large achromatic nucleolus. The maturation divisions were not described.

Arachnida.—Montgomery's ('07) results on *Theridium* are not very extensive from the chromosome-standpoint. He found in the second polar spindle 12 chromosomes and in a fourth cleavage spindle 24 chromosomes. No idiochromosomes were observed.

Excluding the early cleavage groups mentioned above, a number of authors have described the chromosomes of older somatic cells. Henking ('92) found the number of chromosomes in the egg-follicle and connective tissue cells, of *Pyrrhocoris* to be 24. Petrunkewitsch ('01) observed that young blastoderm-cells of the bee contain multiple groups. Miss Stevens ('05*b* and '06*b*) described the somatic groups of several species of Coleoptera and found that the small idiochromosome which occurs only in the male of these forms could be readily identified. Von Baehr ('09) observed that the male somatic groups of *Aphis saliceti* contain 5 chromosomes, one less than the female. Gutherz ('09*a*) concluded that in *Gryllus domesticus* the somatic cells have the same number of chromosomes as the oögonia and spermatogonia respectively, though his observations on this point were not very extensive and no figures of somatic mitoses were given. Doncaster ('10) observed the somatic groups in the male and female pupæ of the gall-fly. He found that in the male, some somatic mitoses show the diploid number of chromosomes while others may show the haploid number. In the female, all somatic mitoses have the diploid number. The very anomalous conditions described for the male do not at present rest upon demonstrative evidence as the chromosomes were found to be small and difficult to count. In addition to the above-mentioned observations, most recent papers on the spermatogenesis of insects contain accounts of the oögonial groups in which idiochromosomes can often be identified.

From the foregoing brief view of the literature on the chromosomes in oögenesis and cleavage, it is evident that with the exception of Miss Stevens and Morgan none of the authors have traced the idiochromosomes into the cleavage and later somatic mitoses, and none but Miss Stevens, Morgan and von Baehr have shown that the embryonic or larval somatic cells of male individuals differ from those of females in the number or size of their chromosomes. Morgan has also shown that idiochromosomes are present in the polar spindle where, in his material, they behave in a characteristic manner. The results on the whole show, I think, that idiochromosomes ("heterochromosomes") are constant chromosome-elements and not merely temporary structures (nucleoli) present during maturation.

Outside of the air-breathing arthropods, there are, as mentioned before, two other groups in which idiochromosomes or similar structures have been found in maturation and cleavage. Baltzer ('08) has found that in two species of sea-urchins there is a particular hook-shaped chromosome which occurs in only a part of the mature eggs. The eggs are thus of two types with respect to this element. (It is replaced by a chromosome of the ordinary sort in the eggs which lack it.) The sperm nuclei on the contrary are all alike. It is not improbable, Baltzer concludes, that the determination of sex depends upon this dissimilarity of egg nuclei, and therefore *lies with the female* (*i. e.*, with the egg), as in the male and female (parthenogenetic) eggs of aphids and phylloxerans. The peculiar hook-shaped elements might thus be called "idiochromosomes." Eggs which contain this element would develop into females, those without, into males. In a very recent paper Boveri and Gulick ('09) have described briefly the chromosome-cycle in *Heterakis*, a nematode. Its cycle corresponds exactly with that of *Protenor* as given by Wilson ('06). The diploid number in the male (spermatogonia) is 9. During spermatogenesis the odd chromosome goes undivided to one pole of the spindle in the first spermatocyte division but divides in the second. The spermatozoa are thus of two classes, with 5 and 4 chromosomes respectively. The diploid number in the female was not determined with certainty but the haploid number in the germinal vesicles and polar spindles was found to be 5. The eggs are thus all alike and, it is assumed, will develop into males or females according as they are fertilized by 4-chromosome or 5-chromosome spermatozoa. The chromosomes of the cleavage nuclei were not described.

Since the results here described for coreid Hemiptera do not give any further insight into the fundamental question of sex-determination but only render the data more complete, it seems needless to add a lengthy discussion on this point. In the recent papers of Wilson, Bateson Castle, Boveri and Morgan, the cytological evidence relating to sex-determination has been thoroughly analyzed. It may be pointed out, however, that apart from theoretical considerations this evidence has been questioned from the standpoint of fact by several workers who have supported their

contentions by direct observations on insect spermatogenesis. Arnold ('08) from his observations on the spermatogenesis of *Hydrophilus piceus* concluded that there were no idiochromosomes in that form, although Miss Stevens has found these elements in all the Coleoptera which she has examined (42 species). The objection offered by Foot and Strobell ('07) to the presence of an odd number of chromosomes in the spermatogonia of *Anasa tristis*, hence of an "accessory" chromosome and the replies to this objection have already been considered. The present work, particularly the section dealing with the cleavage and early blastoderm nuclei, gives further proof that in this species, as well as in the other three examined, the number of somatic chromosomes in the male is one less than in the female. Gross, from his studies on *Syromastes marginatus* ('04) and *Pyrrhocoris apterus* ('06), concluded that the "accessory" chromosome could have no effect on sex-production in these two forms, for he believed that the number of chromosomes is the same in both sexes—22 in *Syromastes* and 24 in *Pyrrhocoris*. In the last named form his counts agree with the earlier ones of Henking ('91), though the latter was uncertain of the spermatogonial number. Recently however Wilson ('09*b*, '09*d*) has reëxamined both these forms and finds that in *Pyrrhocoris*, the male has one less chromosome than the female, *i. e.*, 23 instead of 24, while in *Sryomastes*, the male has 22 as described by Gross but the female has 24 instead of 22. *Pyrrhocoris* may thus be placed in the same class with such forms as *Archimerus*, *Anasa* and *Protenor*. *Syromastes*, however, is unique among the Hemiptera heteroptera in having a bivalent "accessory," though a similar condition has been described by Morgan ('09) in the homopteran, *Phylloxera caryæcaudis*, while Payne ('09) describes several cases among the Reduviidæ (*Fitchia*, *Rocconota*, *Conorhinus*) in which the large idiochromosome (which represents the "accessory") is double.

In addition to the objections cited above, a number of authors have either expressed their doubts of the presence of two sorts of spermatozoa, or, while admitting the existence of such a dimorphism, have questioned its sexual significance. In recent years, however, evidence has been steadily accumulating in support of the conclusion that a nuclear dimorphism of the sperm—

or of the eggs—does occur, not only in insects but in at least two other groups of animals and that it bears a definite relation to sex-production. Whether the sexual tendencies are carried by specific chromosomes or whether certain combinations of chromosomes cause one sex or the other to arise, is still an open question, but that it is possible to demonstrate a nuclear difference in the gametes, and frequently indeed, in the somatic tissues of the two sexes, is now, I think, placed beyond doubt by many decisive observations.

April 27, 1910.

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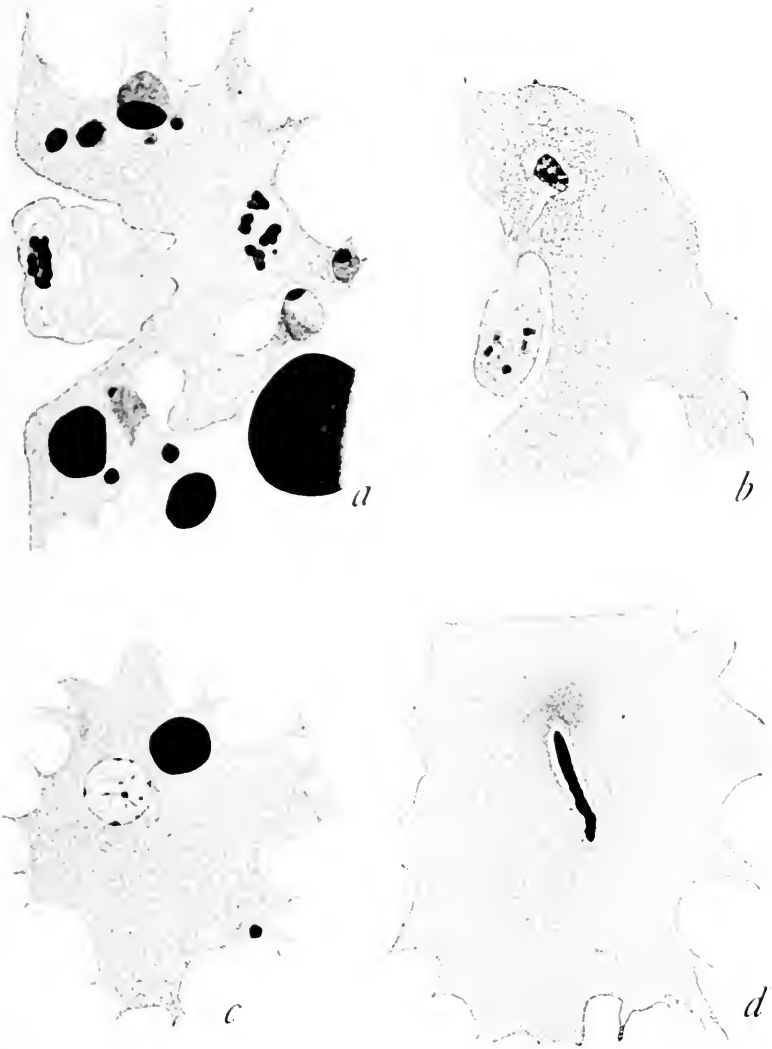
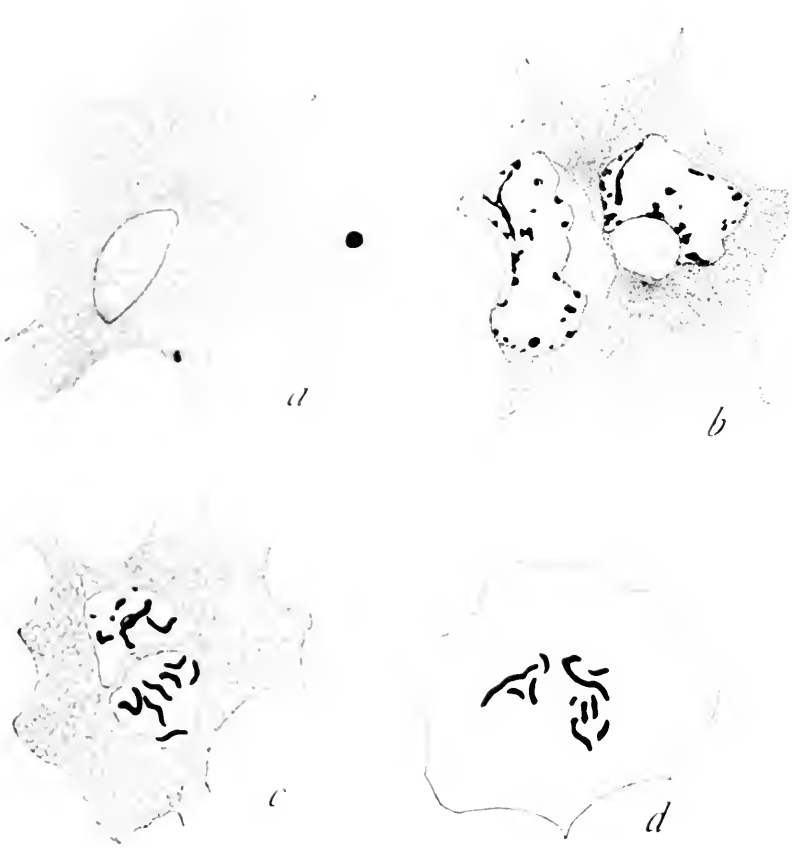
Explanation of Plate I.

Archimerus alternatus. *a*, formation of the first polar body—the mitotic figure is in final anaphase, the inner daughter group showing eight chromosomes; the cytoplasm contains a number of yolk spheres of different sizes and staining capacity. *b*, formation of the second polar body—the inner group of chromosomes have been transformed into the female pronucleus; those of the outer group are still separate. *c*, the female pronucleus advancing into the egg in its cytoplasmic "island"; the latter also contains two yolk spheres. *d*, the sperm-head advancing into the egg, in its cytoplasmic "island"; it is preceded by an aster and surrounded by a clear area. The magnification is 1,375 diameters.


a
b
c
d

Explanation of Plate II.

a–c, Archimerus alternatus. a, a stage letter than Pl. I., *d*—the sperm head has been transformed into the male pronucleus and is still advancing into the egg preceded by its aster and a small clear area. *b*, an early stage in the copulation of the male and female pronuclei—each is in contact with a clear area and an amphiaster lies between them. *c*, a later stage in the copulation of the pronuclei—the nuclear membranes are still intact; seven chromosomes appear in the lower nucleus, the *m*-chromosome being absent; a faint aster appears on the right. *d, Protenor belfragei:* First cleavage prophase, polar view—the male and female groups are still separate, one of them being incomplete; an idiochromosome appears in each group. The magnification is 1,375 diameters.


a
b
c
d

COURTSHIP IN DYSDERA CROCATA.

ALEXANDER PETRUNKEVITCH, Ph.D.

A female and a male of this species of spiders were found under two adjacent flat stones on a sunny hillside in Montclair on the morning of April 21. On the same day they were put in a rectangular small glass dish with a little earth at the bottom, a wire-net lid and a partition of wire-net in the middle to separate the spiders from each other. They at once tried to get at each other, sticking their legs through the net. After a while they desisted and sat quietly on the ground. The next morning the female was found in the corner farthest from the light and next to the screen partition, in a little excavation in the ground, which was protected on all sides with web. The web was destroyed and a fly was held in a forceps before the spider. She bit at it but was either unable or did not want to kill it. The male made no excavation or web. When the partition was removed the male and female met several times in a rather threatening way, opening wide their mandibles and at times seizing each other by the mandibles, but the male would pat the female with his four front feet on the sides and back and then they would separate peaceably. After a short time the female dug rapidly a hole in the ground under a little stone. The male approached several times, touching her legs with his front legs and again going away. I now removed the stone to observe how the female digs the hole. She at once began to work, using all parts of her body. The small clumps of soil she removes by pushing them with palpi, front feet and mandibles with the fangs drawn in. Heavy clumps of the size of her own body she grasps in her fangs and either pushes or pulls backward out of the excavation. The hole is fortified by web which she spins in a very interesting manner. She stands head down with only the abdomen out of the hole and moves the latter in a half circle about the petiolus with spinnerets outstretched, fastening the thread first at one, then at the other end of the half circle. After doing this several times, she turns in the hole to spin the other half circle in the same manner.

Meanwhile the male came quite close and was evidently watching her, at the same time cleaning his legs and palpi by drawing them through his mandibles. After a while he again began his courtship; patted her back, standing face to face and tried to bring his front legs under her body. Later she left the hole without finishing it, returning to it from time to time. They met several times on the ground, always with mandibles wide open and touching each other with the four front legs. At times the female would make a threatening move toward the male upon which he would draw back, but invariably a rapid trembling would seize his four front legs, which lasted from 10–15 seconds. At times when she was out of the hole as though in search of something, he would occuply her place in it. On returning, she gently displaced him. Later as the female showed no intention of accepting the male, I separated them by means of the wire screen.

The next day was cold and rainy and both spiders were found in the morning in separate excavations covered up with earth and web. On the third day after a stormy night it became warm and the glass was placed in the sunshine. The screen was removed and the spiders pushed out of their holes. The male at once went to the female, meeting her face to face. He crept under her sternum and took her firmly by the petiolus between his mandibles with fangs drawn in. With his legs he embraced her in such a way that his right front leg passed between the mandibles (with fangs open) of the female and rested on her back. His left front leg passed between her right mandible and palpus and also rested on her back. The other legs passed similarly between the legs of the female and touched her back. She seemed to make no objection whatever. They tried several times to change their position, the female creeping from place to place while the male continued to cling to her. Finally she lay down on her side and he applied the palpus (at 10.43 A. M.), all the while patting her with his third leg. The coitus lasted five minutes, after which they slowly separated and going to opposite sides of the dish hid themselves in the ground. During the whole time the back of the male was in contact with the sternum of the female, a position not common to the majority of spiders.

In a recent paper on the courtship of araneids Professor Mont-

gomery objects to my conclusion that the sense of sight is the only sense that guides hunting spiders in finding the females during the mating period. He says that he has "frequently noticed males of even diurnal attids and lycosids first recognizing the female by touch." It seems to me however very doubtful that a male should approach a female without having previously noticed her or without having been noticed by her, so to say unawares on the part of both. The fact that the female may stay quiet is no proof that she has not noticed him. From my observations and experiments on hunting spiders I am convinced that they readily see moving objects. If frightened they run away; if conscious of their advantage and hungry or if cornered, they attack. What Montgomery looks upon as recognition by touch may be merely an attempt on the part of the male to find out whether the female is inclined to accept him. I do not deny that such a chance meeting is possible, but from what we know of the habits of spiders, I should expect the female to be either startled or resentful on being touched unawares. In the case of *Dysdera crocata*, which may in a certain sense be regarded as a hunting spider, the male shows every indication of perceiving moving objects and of recognizing the female by sight. Several times he distinctly watched the end of a hatpin with which I was breaking up the hard clumps of earth in a jar. Invariably on my ceasing to do this, he approached the spot and scratched at it with his front legs. As I have already stated he also approached the female whenever she began to dig. Reversing the conclusion of Montgomery that touch occupies the first place in the senses of sex-recognition and sight the second, I repeat therefore that sight is the only sense of sex-recognition in hunting spiders. After sex has been recognized, courtship begins, and touch is the chief means by which the male excites the female and tests her willingness to accept him.

AN ABERRANT LASIUS [FROM] JAPAN.[1]

WILLIAM MORTON WHEELER.

In a small collection of Japanese ants recently sent me for identification by Professor S. J. Kuwana, of the Imperial Agricultural Experiment Station at Nishigahara, near Tokio, I find a single female specimen of such unusual conformation that I at first supposed it to represent an undescribed genus. On closer examination, however, it proves to be a *Lasius* strikingly different from the females of any of the known species, and suggests two hypotheses for both of which provision will be made in the following paragraphs. The specimen may represent either a new species or merely an aberrant female phase of some one of the known Japanese *Lasii*. The latter supposition will be considered at length in the sequel; the former calls for the following, perhaps merely provisional, taxonomic description:

Lasius spathepus sp. nov. (Fig. 1, *A* and *B*.)

Female (deälated). Length 6 mm.

Head cordate, slightly broader than long, with notched posterior border and rounded, convex posterior corners and sides; convex above; gula concave, with a median longitudinal ridge. Mandibles small, flattened; apparently 5-toothed, with concave external borders. Clypeus depressed, broadly rounded in front, obscurely carinate in the middle. Frontal area obsolescent; frontal groove distinct. Eyes rather large; ocelli small. Antennal scapes broad and compressed, reaching well beyond the posterior corners of the head; funiculi slender, not clavate; all the joints distinctly longer than broad; joints 1–3 more than twice as long as broad; terminal twice as long as the penultimate joint. Thorax much narrower than the head, fully twice as long as broad; mesonotum and scutellum flattened above; epinotum short, rounded above, with the declivity abrupt, straight in profile and longer than the base. Petiole with an erect scale, compressed anteroposteriorly and with its upper margin rather sharp and distinctly notched in the middle. Gaster very short, but little longer than

[1]Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 22.

broad. Anal papilla prominent. Legs very long; femora, tibiæ and metatarsi dilated and compressed anteroposteriorly; remaining tarsal joints growing successively narrower.

Body and appendages smooth and shining, very finely and inconspicuously punctate. Pleuræ and especially the sides of the epinotum more opaque and somewhat more coarsely punctate. Mandibles opaque, finely and sharply striated.

Hairs yellowish, very short and sparse on the body, denser and more appressed on the flat surfaces of the legs, but absent on the

FIG. 1. *A*, *Lasius spathepus* sp. nov., dealated female; *B*, head of same.

sharp dorsal and ventral edges of these appendages. Anterior border of clypeus with a row of short, stout bristles. Border of petiole and posterior edge of each gastric segment with a single row of short hairs. Circlet of anal cilia long and coarse.

Body deep chestnut brown; scapes, legs and articulations of wings paler and more reddish; corners of clypeus and posterior borders of gastric segments sordid yellow.

This female may be at once distinguished from any of the known female *Lasii* by its peculiar heart-shaped head, short gaster and dilated and flattened metatarsi. The last character, in fact, is not met with in any other known ant, except *Melissotarsus*, which Emery regards as an aberrant Ponerine.

The supposition that *L. spathepus* may not be a new species, but merely an extraordinary female form of some one of the well-known Japanese *Lasii*, is supported by the following considerations. Many years ago Walsh[1] described an aberrant female

[1] "On the Genera of Aphidæ found in the United States," *Proc. Ent. Soc. Phila.*, I., No. 9, 1862, pp. 204–311.

Lasius from Illinois as *L. latipes*, and in 1903 McClendon and I[1] showed that this ant has two forms of females: the one described by Walsh and characterized by extremely flat, dilated femora and tibiæ, small, feeble tarsi, strongly clavate antennal scapes, short funicular joints and long, fulvous pilosity; and another of a darker color, with less flattened legs, less clavate scapes, longer funicular joints, longer tarsi and sparser, shorter pilosity. The latter we designated as the α-, the former as the β-female. We found most colonies at the height of the breeding season to contain only β-females, but in three colonies from different localities both forms occurred simultaneously. These observations suggest that *L. spathepus* may be the β-female of some Japanese *Lasius*, which in its worker and male phases shows no departure from the usual generic type of structure. Five *Lasii* are known from Japan, namely, *L. niger* L., *niger alienus* Förster, *myops* Forel, *umbratus* Nyl. and *L. fuliginosus* Latr. All of these are well-known European species and, in all probability, common also throughout temperate Asia.[2] The only one of these species of which *spathepus* could be a β-female is *L. fuliginosus*. I possess males and workers of this species collected by Mr. Hans Sauter in Kanagawa, Japan, and there were three workers in the collection sent me by Professor Kuwana, but as these bear a special number they were probably not taken in the nest containing the *spathepus*. All the Japanese workers and males of *fuliginosus* are indistinguishable from specimens in my collection from several European countries (England, France, Germany, Switzerland, Austria and Russia). In Europe, however, this ant is known to have only one form of female, which is in no respect extraordinary (Fig. 2, *A* and *B*) though it would bear to *spathepus* about the same relation that the α-female of *latipes* bears to the cospecific β-female. Comparison of the figures accompanying this article shows that the head of *spathepus* in its outline is in some respects more like that

[1] "Dimorphic Queens in an American Ant (*Lasius latipes* Walsh)," BIOL. BULL., IV., No. 4, 1903, pp. 149–163.

[2] *L. fuliginosus* is cited by Forel from lower altitudes in the Himalaya ("Les Fourmis de l'Himalaya," *Bull. Soc. Vaud. Sc. Nat.*, 5 ser., XLII., 1906, p. 85). Du Buysson in a paper which I have not seen ("Les fourmis fuligineuses au Japon," *Rev. Ent.*, 1906, pp. 101–102) gives some notes on the occurrence of this ant in Japan.

of the worker *fuliginosus* than the latter is like that of the European female of the species. If *spathepus* is an aberrant female *fuliginosus*, as seems not only possible, but probable, we must therefore assume either that this species in Japan has two females, comparable to the α- and β-females of *latipes*, or that it has only the β-female, while the α-female alone is retained in Europe

FIG. 2. *A*, *Lasius fuliginosus* Latr., dealated female; *B*, head of same; *C*, head of worker.

Evidently this question can be decided only by exhaustive observations in Japan.

The train of hypotheses suggested by *spathepus* is not terminated at this point. Recent investigations make us look with increasing interest on all aberrant female ants, for it has been found that certain species of *Formica*, *Aphænogaster* and *Bothriomyrmex* which have females either of unusually small size, glabrous integument, extraordinary color or pilosity, or with unusual morphological characters, also exhibit correlative ethological peculiarities. Such females, during the establishment of their formicaries, are, as a rule, temporary parasites on workers of allied species whose females retain the typical generic characters. The question therefore arises as to whether the aberrant female, which I have called *L. spathepus*, may not be a temporary parasite on some other more common species of *Lasius*. Here the suggestion that *spathepus* may be a β-female of *fuliginosus* receives a little support

from some recent European investigations. Forel[1] long ago showed that this ant is unique among the old world *Lasii* (and the new world species may be included in this statement) in its odor, the great size of its colonies, its habit of foraging in long files in the broad day-light and in constructing carton nests in old tree-trunks. Wasmann[2] has recently called attention to its ability to form new colonies by sending off detachments of queens and workers after the manner of *Formica rufa*. Like *rufa* it also possesses another method of colony formation, namely, through temporary parasitism. Unlike the queens of the common *Lasius niger*, the queen of *fuliginosus*, after fecundation on her marriage flight and on returning to the earth, is unable to start a colony unaided, and if prevented from rejoining the maternal colony or a detachment of workers of her own species, has to seek out a colony of *L. umbratus* and have her young brought up by the workers of this ant. The *umbratus* queen must be killed either by her own workers or by the intrusive *fuliginosus* queen, so that the host species is destined eventually to die off and leave a pure and thriving *fuliginosus* colony. That this method of colony formation is actually adopted by *fuliginosus* queens is clearly indicated by the following observations which have been slowly accumulating during the past few years: In 1908 de Lannoy[3] stated that in 1904 he found at Knoche-sur-Mèr in Belgium a few workers of *L. mixtus* (a subspecies of *umbratus*) living in a large colony of *fuliginosus*, and that in 1906 he found several similar colonies. Emery[4] and Forel[5] interpreted these observations to mean that the queen *fuliginosus* founds her colony with the aid of *umbratus* workers, in a manner analogous to that employed by the North American and European *Formicæ* of the *rufa*, *exsecta* and

[1] "Les Fourmis de la Suisse," *Nouv. Mém. Soc. Helv. Sc. Nat. Zürich*, XXVI., 1874, pp. 1–447, 2 pls.

[2] "Ueber gemischte Kolonien von Lasius-Arten," *Zool. Anzeig.*, XXXV., 1909, pp. 129–141.

[3] "Notes sur le *Lasius niger* et le *Lasius fuliginosus*," *Ann. Soc. Ent. Belg.*, LII., 1908, pp. 47–53.

[4] "Remarques sur les observations de M. de Lannoy touchant l'existence de *L. mixtus* dans les fourmilières de *L. fuliginosus*," *Ann. Soc. Ent. Belg.*, LII., 1908, pp. 182, 183.

[5] "Lettre à la Société Entomologique de Belgique," *Ann. Soc. Ent. Belg.*, LII., 1908, pp. 180, 181.

microgyna groups when they enter nests of *F. fusca* and *incerta*. Wasmann (*loco citato*) accepts the interpretation given by Emery and Forel, and now recalls that he has on several occasions found mixed colonies of *L. umbratus* and *fuliginosus*. Donisthorpe[1] states that in 1897 he recorded the occurrence of a large colony of *fuliginosus* in a hollow tree at Lymington, England, living with what he believed at the time to be *L. flavus* but has since decided must have been *umbratus*. He also says that Crawley has recently found *umbratus* workers in company with *fuliginosus*.

But even this is apparently not the whole story. Crawley[2] found that the queen of *umbratus* may be adopted by a colony of *L. niger*, and Wasmann (*loco citato*) has shown that the former ant is, at least occasionally, a temporary parasite on *niger*, for he found a mixed colony of the two species which could only be interpreted on this supposition. He believes, therefore, that we may have here a case of social hyperparasitism—*umbratus* founding its colonies with the aid of *niger*, and *fuliginosus* with the aid of *umbratus*.' In these observations it is, of course, the female of the European *fuliginosus* which exhibits temporary social parasitism, and if *spathepus* is really the ♂-female of this species, it is also, in all probability, addicted to the same form of parasitism, perhaps on some other species of *Lasius*, although *umbratus*, as I have stated, is known to occur in Japan.

Thus it appears that in the old world the genus *Lasius*, like the genus *Formica*, is made up of two sets of species—one extremely abundant and widely distributed, with queens able to establish their colonies independently, the other rare and sporadic in their occurrence, with queens that require the assistance of workers of other species of the genus when engaged in founding their commonwealths. To the former set belong *L. niger* L. and its various subspecies (*alienus* Förster, *brunneus* Latr., *emarginatus* Fabr., *lasioides* Emery) and *L. flavus;* to the latter *L. umbratus* Nyl., and its subspecies *mixtus* Nyl., *bicornis* Förster and *affinis* Schenck, *L. carniolicus* Mayr and *fuliginosus* Latr. The great rarity of *carniolicus* and the very small size

[1]"On the Founding of Nests by Ants; and a few notes on Myrmecophiles," *Ent. Rec.*, XXII., No. 3, 1910, 4 pp.

[2]*Ent. Month. Mag.*, 1909, p. 94.

of its females point unmistakably to parasitic habits. The same is probably true of *L. crinitus* described by F. Smith[1] and Mayr[2] from Cashmir. Only the female of this species is known and this has long yellow hairs like the North American *Formica ciliata* Mayr and *crinita* Wheeler, which are, in all probability, temporary parasites on varieties of *F. schaufussi* or *fusca*.

In North America, the genus *Lasius*, which embraces the subgenus (*Acanthomyops*) not represented in Eurasia, seems to present a corresponding division of its species into those with independent and those with parasitic queens, although the data on which this assertion is based are at present very meager. Here, too, the forms of *L. niger*, namely the varieties *americanus* Emery and *neoniger* Emery, *L. flavus* var. *nearcticus* Wheeler and *brevicornis* Emery establish their colonies independently. This I can affirm from many observations in the field. The same is true of *L.* (*Acanthomyops*) *claviger* Roger and probably also of *L.* (*A.*) *interjectus* Mayr. But I have never seen any of the females of our *umbratus* forms (*mixtus* var. *aphidicola* Walsh, *subumbratus* Viereck, *minutus* Emery and *speculiventris* Emery) in the act of founding their colonies independently, and it is quite probable that they are temporary parasites on the extremely common *L. americanus*. Equally negative have been my observations on *L* (*A.*) *latipes*, which has the α- and β-females to which I alluded in a preceding paragraph. That this species is a temporary parasite on *L. americanus* is indicated by the fact that near Colebrook, Conn., I found four small mixed colonies.[3] *L.* (*A.*) *murphyi* Forel and *occidentalis* Wheeler, which are closely related to *latipes* and have females covered with singular fulvous hairs, are also very probably to be regarded as parasitic in the early stages of colony formation.

The genus *Lasius*, which comprises some of the commonest and most characteristic ants of the north temperate zone, has never attracted a large number of students, probably because the

[1] "Catalogue of Hymenopterous Insects in the Collection of the British Museum," Pt. VI., Formicidæ, 1858, p. 13.

[2] "Myrmecologische Studien," *Verh. K. K. Zool. bot. Ges. Wien.*, XII., 1862, p. 700.

[3] See Wheeler, "Ants: Their Structure, Development and Behavior," 1910, p. 504, *nota*.

species are in the main subterranean and are so much more monotonous in their habits than the species of *Formica*. Nevertheless, an intensive study of the ethology of the European and North American *Lasii* is bound to bring to light many surprising facts. This is sufficiently indicated in the preceding paragraphs notwithstanding the large amount of conjecture which they contain.

SPERMATOGENESIS OF THE MYRIOPODS.

VI. An Analysis of the Chromosome Group of *Scolopendra heros*.[1]

M. W. BLACKMAN.

During the last few years a number of attempts have been made to analyze the chromosome complex of various animals. These attempts have met with such apparent success in the case of several insects, notably Orthoptera, that I have been led to attempt a similar analysis of the chromosome group in *Scolopendra heros*. Indeed, before the appearance of earlier papers upon this species such an attempt had been made, but it had met with but small success owing, as I now know, to deficiencies in the optical apparatus employed. With the facilities then at my disposal, it was impossible to secure definite clear-cut images of the chromosomes at a magnification greater than 1,500 diameters. As the chromosomes in *Scolopendra heros*, although exceedingly clear-cut and definite in outline, are considerably smaller than in some insects, a greater magnification than 1,500 diameters is necessary if the study is to be at all convincing, either to the investigator or to those reading his report. However, by the use of a Zeiss 2-mm. apochromatic objective and a number 12 compensating ocular, the source of light being a Welsbach mantle, a magnification of 2,300 diameters was obtained with no perceptible loss of definition in the image.

The material used in this study is the same which served as a basis of several previous papers[2] (Blackman :01, :03, :05), the

[1]From the Zoological Laboratory, Syracuse University, Syracuse, New York.

[2]Blackman, M. W., :01, "The Spermatogenesis of the Myriapods—I., Notes on the Spermatocytes and Spermatids of *Scolopendra*," *Kans. Univ. Quart.*, Vol. 10, pp. 61–76, pl. 5–7.

Blackman, M. W., :03, "The Spermatogenesis of the Myriapods—II., On the Chromatin in the Spermatocytes of *Scolopendra heros*," Biol. Bull., Vol. 5, pp. 187–217, 22 fig.

Blackman, M. W., :05, "The Spermatogenesis of the Myriapods—III., The Spermatogenesis of *Scolopendra heros*," *Bull. Mus. Comp. Zool., Harvard Coll.*, Vol. 48, No. 1., pp. 1–137, 9 pl.

majority of the slides having been mounted nine years, but the stain (Heidenhain's iron-alum hæmatoxylin), except where a portion of the section extends from under the cover-glass, is as perfect as when first mounted.

Sutton,[1] :02, Robertson,[2] :08, and Nowlin,[3] :08, working upon the male cells of Orthoptera have found that the chromosomes during both the spermatogonial and spermatocyte generations may be arranged in a graded series as regards size. In the spermatogonia this series is a double one, the two chromosomes of a given size representing the similar elements derived from the two parents. These similar chromosomes unite during synapsis (Sutton, :02, *op. cit.*), and give rise to the single series characteristic of the spermatocytes. The extreme difference in the size of the chromosomes in the Orthoptera is so marked that it is noticeable at a glance (see Sutton, :02, Fig. 6) and after studying preparations of this material one cannot doubt the accuracy of their observations or deny the strength of their conclusions, that in these forms the chromosomes at any given stage bear a certain size relation to each other, and that this presents strong evidence in support of the theory of the individuality of the chromosomes.

But if we grant these conclusions with regard to the forms studied, does it necessarily follow that these conclusions should be made more general and applied to the chromosomes of all animals? What shall we say as regards the application of this test to the chromosomes of a form in which the difference in size is not so marked or in which the chromosomes all appear of nearly one size? Such is apparently the case in *Scolopendra*. At ordinary magnification there is very little difference in the size of the chromosomes as seen in a metaphase of the first spermatocyte. *Some* difference is to be detected even at a magnification as low as 1,000 diameters, but this is so slight that if size alone be used as a criterion it would seem impossible to distinguish between the chromosomes farther than to say: "This is one of the smaller ones" or "one of the larger ones."

[1]Sutton, W. S., :02, "On the Morphology of the Chromosome group in *Brachystola magna*," BIOL. BULL., Vol. 4, pp. 24–39, 11 fig.

[2]Robertson, W. R. B., :08, "The Chromosome Complex of *Syrbula admirabilis*," *Kansas Univ. Sci. Bull.*, Vol. IV., pp. 275–305, 5 plates.

[3]Nowlin, Nadine, :08, "The Chromosome Complex of *Melanoplus bivittatus* Say," *Kans. Univ. Sci. Bull.*, Vol. IV., pp. 265–271, 2 plates.

But other tests may be applied and have been applied. Baumgartner[1] (:04), made an attempt to distinguish the chromosomes in *Gryllus* by differences in form. He reaches the conclusion that in *Gryllus* certain definite shapes constantly occur and establishes the probability that there is a fixed number of each type. Davis[2] (:08), working upon various Orthoptera reaches the conclusion that "In addition to the difference in volume, the bivalent autosomes (chromosomes) show constant and characteristic differences in form. In general several more or less distinct morphological types can be distinguished, and the members of each type appear to bear a constant numerical relationship to each other."

Robertson, :08 (*op. cit.*), does not consider the shape of the chromosome of first importance in establishing its identity but considers size as the primary characteristic, while shape is secondary and to a certain extent dependent upon size or at least upon the degree of lengthening. The main criticism I wish to make regarding Robertson's conclusions on this point is that in his study of the chromosomes, he has not drawn them from the best view point to establish any characteristic difference in shape. His drawings are all or nearly all of chromosomes as seen in polar view, whereas a view at right angles to the spindle is more satisfactory in determining both the shape of the chromosomes and their relation to the mantle fibers.

In *Scolopendra*, as I have already implied, it is impossible to establish the identity of many of the chromosomes on the basis of size alone. Early in my work, however, after six or eight chromosome groups had been carefully drawn, it became evident that the chromosomes as seen in a lateral view of the metaphase of the first spermatocyte are of several distinct types as regards shape and that the size relation of the chromosomes of each type are such as to make it possible to distinguish the individual chromosomes with some degree of certainty. This, I think, will be apparent from a study of the figures of plates I. and II., although it must be borne in mind that the figures are of course much less satisfactory for this comparison than the actual chromosomes,

[1]Baumgartner, W. J., :04, "Some New Evidences for the Individuality of the Chromosomes," Biol. Bull., Vol. 8, pp. 1–23, 3 plates.

[2]Davis, H. S., :08, "Spermatogenesis in Acrididæ and Locustidæ," *Bull. Mus. Comp. Zoöl. Harvard Coll.*, Vol. LIII., pp. 59–158, 9 plates.

due to the fact that many of the chromosomes do not lie at right angles to the line of vision and must, therefore, appear foreshortened in an outline drawing.

Before discussing the individual characteristics of the various chromosomes as seen in metaphase, it might be well to give a brief review of their history in the prophase. The spermatocyte chromosomes are seventeen in number. Of these, sixteen are bivalent elements formed by the end to end union of univalent chromosomes during the telophase of the last spermatogonial division (Blackman, '03, '05, *op. cit.*). The seventeenth element, the accessory chromosome, is univalent in character, being derived directly from a single specialized chromosome, the accessory chromosome of the spermatogonium. The growth period following synapsis is of long duration in *Scolopendra*, and during this period all of the chromosomes are grouped together to form a nucleolus-like structure to which I have given the name karyosphere. While in the karyosphere the chromosomes are so closely aggregated that their individual outlines cannot be distinguished with certainty. They, however, enter the karyosphere as distinct individuals and later arise from it as definite chromatin segments, similar in every respect except for their greater size. These facts would seem to argue for, rather than against, the individuality of the chromosomes during this stage.

In the prophase the chromosomes arising from the karyosphere are typically long, slender threads of granular chromatin, which invariably show near their middle an interruption of the chromatin—this representing the point at which the chromosomes united during synapsis. The two spermatocyte divisions always result in a longitudinal and a cross division of these bivalent elements. The longitudinal division as a rule seems to occur first, although, as we shall see later, this is not invariable, even for the ordinary chromosomes. The cross division or reduction division results in the separation of entire spermatogonical chromosomes, the division occurring at the point at which they united during synapsis. However, although the results of these divisions are the same for all of the chromosomes (with the exceptions to be noted later), the changes through which the tetrad pass in the prophase and the shapes they assume during the pro-

phase and later during the metaphase differ to some extent. As this difference in shape is one characteristic by which we must hope to identify the various chromosomes, occasion may be taken here to describe briefly the processes by which these various forms arise.

What I shall call type A is represented in the text-figure I. The origin and evolution of this type of tetrad is described in sufficient detail in previous papers (Blackman, :03, :05, *op. cit.*); so it will not be necessary to repeat the description in detail.

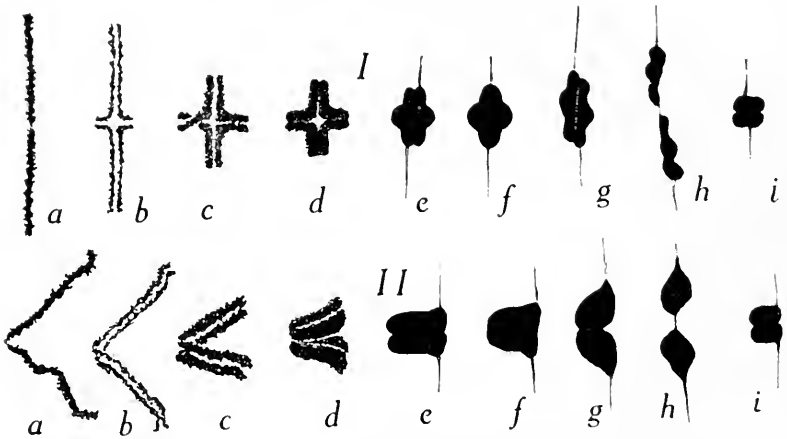


FIG. I. Semi-diagrammatic representation showing the formation and history of the cross-shaped type of tetrads; *a*, bivalent chromatin segment as it appears in the very early prophase; *b*, planes of longitudinal and transverse cleavage established; *c*, *d*, later stage in evolution of prophase tetrad; *e*, *f*, tetrads as seen in early metaphase; *g*, tetrad in act of division, showing the manner in which the component parts glide over each other; *h*, early anaphase showing distortion of halves of tetrad due to their close adhesion; *i*, daughter chromosome in metaphase of second spermatocyte.

FIG. II. Corresponding stages in evolution of the double-V type of tetrad.

I wish, however, to emphasize two points. First the points at the ends of the shorter arms of the cross-like figure (Fig. I, *b*, *c*, *d*) represents the point at which union occurred during synapsis. The attachment of the mantle fibers in the metaphase is *not at this point* as it is said to be in *Syrbula* by Robertson (:08, *op. cit.*), but is at the ends of the longer arms of the cross as shown in Fig. I, *e*, *f*, *g*.

Robertson believes that the attachment of the mantle fibers

coincides with the point of synaptic union of the elements and that each bivalent chromosome during its division undergoes a "change of its long axis from a longitudinal to a transverse direction." This is accomplished by a rotation of the chromatids over each other in such a manner as to result in a longitudinal division of the tetrad in the first spermatocyte. In *Scolopendra*, as I have shown in previous papers (*op. cit.*), no such complicated process occurs in division. The long axis of the tetrad in most cases remains parallel to the line of longitudinal cleavage and in the metaphase the two halves glide over each other during the act of division. As may be seen in the semi-diagrammatic drawings (Fig. I, *g*, *h*) and in several of the chromosomes of this type in the accompanying plates, the two halves of the tetrad seem to adhere rather closely and there is often considerable distortion. In Fig. I, *h*, drawn from my preparations direct it will be seen that the parts of the two daughter chromosomes remaining longest in contact are considerably lengthened and distorted apparently due to the firm adhesion of the two parts.

The second type of spermatocyte chromosome is the "double-V" tetrad described by me in a previous paper (*op. cit.*). This type usually arises from the bivalent chromosomes of the early prophase which are bent at a sharp angle at the point of synapsis. After the longitudinal cleavage of the chromatin thread has occurred the double thread becomes shorter and thicker, resulting in the double-V-shaped structure shown in Fig. II, *c*. There is at all times a very apparent interruption of the chromatin at the angle of each thread (point of synapsis), and it is at this point that the cross division occurs later. In the late prophase there is a still further condensation of the chromatin and shortening of the thread, resulting in the closer apposition of the ends of the threads farthest from the point of synapsis, resulting in a chromosome of the shape shown in Fig. II, *e*, *f*. At the time of the formation of the spindle the mantle fibers come to be attached to the distal ends (ends farthest from point of synapsis) of the tetrad in such a manner (Fig. II, *e*, *f*, *g*) that the chromosome is divided along its longitudinal axis. In this type also, the two halves of the chromosome seem to adhere closely and to divide reluctantly (Fig. II, *g*, *h*, also Figs. 6, *i*, and 17, *j*).

The chromosomes of the third type arise from thread-like structures similar to those from which type A and B arise. This thread may be either approximately straight, or it may be curved slightly in various ways, but is never bent at a sharp angle at the point of synapsis. The filament undergoes a longitudinal cleavage just as with the other types. The two resulting threads, as a usual thing, lie parallel to each other (Fig. III, *b*, *c*) but in some

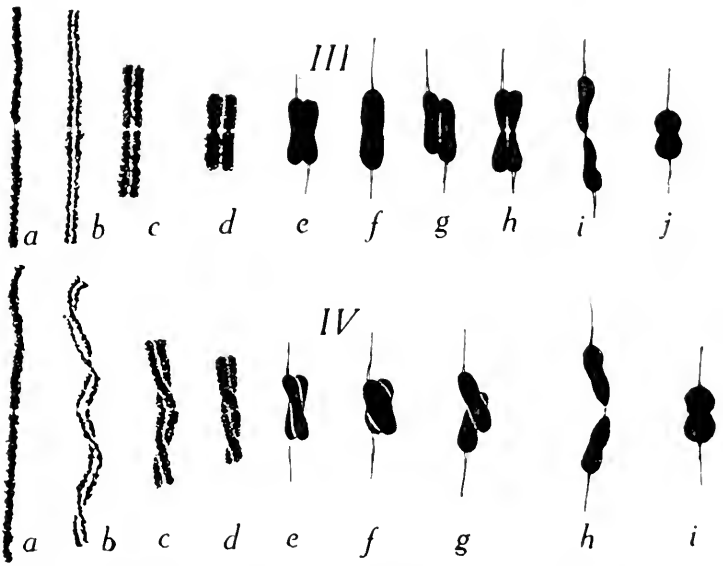


FIG. III. Evolution of the double-rod-shaped tetrads. *a*, bivalent chromatin segment; *b*, *c*, *d*, formation of tetrad; *e*, *f*, tetrads as seen in early stages of the spindle; *g*, *i*, ordinary tetrad in two stages of longitudinal division; *h*, rod-shaped tetrad apparently in act of transverse division; *j*, dyad as seen in metaphase (secondary spermatocyte).

FIG. IV. Variation of double-rod-shaped tetrad. In early prophase the double chromatin segment is often twisted as shown in *b*. The shortening of chromatin thread results in less and less twisting; so that the two parts of the metaphase chromosome merely overlie each other at an angle or are only partially wrapped about each other.

cases they are twisted about each other, so as to form a rope-like structure (Fig. IV, *b*, *c*). In such cases the resulting chromosome has a somewhat different shape. In this type of chromosome the tetrad resulting appears rod-shaped or double rod-shaped depending upon the angle from which the structure is viewed.

After the planes of longitudinal and cross division are established, the further changes involve merely a shortening and condensation of the entire structure. In Fig. III, *c*, *d*, two stages in this process are shown, both cleavages being very evident. Later, as the condensation continues these planes of cleavage are obscured to some extent and usually the only evidence we have of the longitudinal cleavage is a very definite notch at each end of the chromosome, while the plane of cross division is usually shown merely by a slightly lessened diameter of the tetrad near its middle (Fig. III, *d*, *e*, *f*). In some cases, however (Fig. 3, *g*, *h*), the planes of longitudinal and transverse cleavage may be seen very distinctly even in the metaphase (Fig. 4, N, 13, *n*, 16, *n*, etc.). In metakinesis a longitudinal division is accomplished by a gliding apart of the two halves of the tetrad (Fig. III, *g*, *i*) in a manner essentially similar to the division of the cruciform tetrads.

In a number of the cells studied there seems to be one of the chromosomes of this type, which presents quite a different appearance during the act of division. A constriction appears in this chromosome at about the middle point (the plane of transverse cleavage). This is so pronounced that there is in many cases (dependent on the stage of division) a partial or even a complete interruption of the chromatic material at this point (Figs. 3, N, 4, N, 9, N, 10, N, etc.). From a careful study of this chromosome in many cells, the conclusion seems inevitable that this one tetrad undergoes a transverse division, while the rest of the chromosomes are dividing longitudinally.

A variation of the rod-shaped tetrad is shown in text-fig. IV. Here after the longitudinal cleavage has been established, the two threads, instead of lying side by side, are twisted about each other in such a way as to form a rope-like structure. The resulting metaphase chromosome differs somewhat in appearance from others of this type, although in all essentials it is identical. As the threads shorten the twisting gradually becomes less and less pronounced (Fig. IV, *b*, *c*, *d*, *e*) until in the completed chromosomes there is only a slight twisting and usually the two parts merely overlie each other at an angle. The division accomplished by the first maturation mitosis is a longitudinal one.

Besides these three types of ordinary chromosomes all of which

are bivalent there is one element which is univalent in character, being derived directly from a single spermatogonial chromosome. This is the accessory chromosome and in the metaphase can always be distinguished by its characteristic shape, and especially by the fact that it is connected by mantle fibers to only one pole of the spindle. There is usually no indication of the plane of division in the accessory chromosomes at this time, although in the prophase a longitudinal constriction is often shown.

The chromosomes of *Scolopendra heros* are seventeen in number, sixteen of which are bivalent, while one is univalent. The sixteen bivalent chromosomes undergo longitudinal and transverse divisions during the prophase and at the beginning of the metaphase are of several different types as regards shape. After studying a large number of metaphases of both the large and small (Blackman, '05) type of spermatocytes it has been found to be a rule that in each cell the chromosomes of the several types bear a definite and constant numerical relationship to each other. This fact can best be appreciated by referring to the accompanying plates. It will be seen at a glance that the cruciform tetrads which have been described above as type A are in all cases six in number. Furthermore, among the chromosomes of this typical form a definite size relation exists which makes it possible to arrange the cross-shaped chromosomes in a graded series on the basis of bulk. To be sure, the difference in size is not so striking as that existing between the largest and smallest chromosomes in some insect material, but I believe is as great as the difference in size between adjacent chromosomes of the graded series in insects. It is perhaps unnecessary to explain that the *actual* size difference is in many cases greater than appears in the camera lucida drawings, owing to the fact that some of the chromosomes are foreshortened on account of the angle from which they are viewed. For this reason, the drawings are much less convincing than the preparations.

The shape of the chromosomes, aside from showing an apparent modification due to the angle of vision, actually does vary considerably but each of the group of six chromosomes in question are always reducible to the cruciform tetrad described as type A. The variations in shape have to do only with the degree to which

the short arm of the cross is drawn out and to apparent differences incident to the angle of vision. When the short arm of the cross is drawn out but little the tetrad approaches the rod-shaped chromosomes of type C. Then, too, the cruciform tetrads vary in shape in different stages of the metaphase. The limbs of the cross are more likely to be of nearly equal size in the early spindle stages than when division has actually begun. As the chromosomes even in the same equatorial plate, are not all in exactly the same stage of division at the same time this factor should receive consideration.

An attempt was made to learn whether any of the chromosomes constantly either lag behind or precede the others in division. It was found that chromosome A, the largest of the cross-shaped tetrads, shows a tendency to lag somewhat behind the others of this type. In many of the chromosome groups this is very evident. In some cells it is not yet oriented in the characteristic manner in the equatorial plate at a time when some of the others have begun to show the characteristic change in shape incident to the gliding apart of their component elements. This can be seen by comparing chromosome A with other chromosomes of similar type in Figs. 1, 3, 4, etc. Then, too, this chromosome often presents a less clear-cut outline than do the others, approaching the granular condition characteristic of the prophase.

Of the chromosomes of type B there are five present in the metaphase of *Scolopendra heros*. This is the type of chromosome which shows the characteristic double-V shape in the prophase. The difference in shape between the tetrads of this type and the cross-shaped elements is usually quite striking. Even more characteristic of this type is the attachment of the mantle fibers and the orientation of the chromosomes in the equatorial plate. As seen in side view, the chromosome is more or less rectangular in shape, but one end is usually wider than the other and to the angles of this end the mantle fibers are attached. The chromosome usually lies with this end toward the center of the spindle, while the free end (that to which the mantle fibers do not attach) extends outward. This free end is often notched and this notch indicates the plane of longitudinal division. In end view (Figs. 1, *I*, 6, *J*, etc.) the appearance is not so characteristic in the draw-

ings, although in the preparations there is no difficulty in recognizing the true shape of the chromosome, the apparent difference in shape being due to the view-point from which it is seen.

The five chromosomes of type B form a graded series as regards size, just as with those of type A. The largest one is very perceptibly greater than the smallest, and the intermediate ones differ in size to such an extent that there is usually little difficulty in assigning them to their proper place in the series. No individual of this type shows any constant precocity or tardiness in division, although in some cells one or more of them are farther along than the others (Figs. 6, *I*, 17, *J*).

The rod-shaped tetrads (type C) are five in number in *Scolopendra heros*. They show the same constancy in size relation as do the other types, and may be readily arranged in a graded series. Usually one or more of this type of chromosome show the component parts overlapping each other at an angle or partially wrapped around each other, indicating that they arise from the twisted threads often seen in the prophase and already described. However, these are not constant in occurrence and this condition seems to depend largely upon chance.

A fact which has proved rather puzzling was brought out by a careful study of the various chromosomes of this type. While it cannot be doubted that four of the rod-shaped chromosomes divide longitudinally in the first spermatocyte division, the fifth tetrad of this shape apparently divides transversely. In all cases in which this element is well advanced in the metaphase there is a very evident constriction at its middle point, and in some cases this amounts to a nearly complete interruption of the chromatic material. This is especially evident in Figs. 3, 4, 10, chromosome *N*. Indeed, it seems hardly possible to escape the conclusion that at the same time the other fifteen bivalent chromosomes are undergoing longitudinal division, this one element is divided reductionally.

This, however, is no less to be expected than is the behavior of the accessory chromosome in this same division. It differs from the other chromosomes in being univalent (*i. e.*, it has no synaptic mate), while the rest are bivalent. After the formation of the spindle it lies among the other chromosomes and is scarcely distinguishable from the rod-shaped ones aside from the fact that

it is connected by mantle fibers to only one pole of the spindle. It is not divided by the first spermatocyte division but passes to one pole entire. Thus the result is in a sense similar to a reductional division, the two cells differing as regards the distribution of this element. It has no synaptic mate and is, therefore, distributed to only one of the resulting cells (Figs. 19, 20).

It is evident that the unequal distribution of the accessory chromosomes produces two sorts of secondary spermatocytes—one half having only the sixteen ordinary chromosomes and one half having the accessory chromosome in addition. By the second spermatocyte division, the accessory chromosome is divided and as it occurs in but half of the second spermatocytes it is distributed to only half of the spermatids, thus giving rise to a dimorphism among the spermatids and spermatozoa. The significance of this dimorphism has been discussed by a number of investigators—McClung[1] (:02), Wilson[2] (:06), Stevens[3] (:08, :08*a*, :09) Boring[4] (:07) and others—and, as I have nothing new in the way of observations to offer it would appear hardly profitable to consider the subject in detail. I believe, however, that when the chromosomes of the female germ cells of *Scolopendra* are studied it will be found that these are thirty-four in number in the ovogonia, and that the following fertilization formulæ of Wilson (:06, *op. cit.*) will hold good for this species:

$$\text{Egg } \frac{N}{2} + \text{Spermatozoön } \frac{N}{2} \begin{pmatrix} \text{including} \\ \text{accessory} \end{pmatrix} = N \text{ (female)}.$$

$$\text{Egg } \frac{N}{2} + \text{Spermatozoön } \frac{N}{2} - 1 \begin{pmatrix} \text{accessory} \\ \text{lacking} \end{pmatrix} = N - 1 \text{ (male)}.$$

[1]McClung, C. E., :02, "The Accessory Chromosome—Sex Determinant?" Biol. Bull., Vol. 3, pp. 43–84.

[2]Wilson, E. B., :06, "Studies on Chromosomes—III., The Sexual Differences of the Chromosome Group in Hemiptera, with some Considerations on the Determination and Inheritance of Sex," *Journ. Exp. Zoöl.*, Vol. III., pp. 1–40, 6 fig.

[3]Stevens, N. M., :08, "A Study of the Germ Cells of Certain Diptera with Reference to the Heterochromosomes and the Phenomena of Synapsis," *Journ. Exper. Zoöl.*, Vol. V., pp. 359–374, 4 plates.

Stevens, N. M., :08*a*, "The Chromosomes in *Diabrotica vittata*, etc.," *Journ. Exper. Zoöl.*, Vol. V., pp. 453–469, 3 plates.

Stevens, N. M., :09, "Further studies on the Chromosomes of Coleoptera," *Journ. Exper. Zoöl.*, Vol. VI., pp. 101–113. 4 plates.

[4]Boring, Alice M., :07, "A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ," *Journ. Exp. Zoöl.*, Vol. IV., pp. 469–512, 9 plates.

In *Scolopendra* (Blackman, :05, *op. cit.*) there are two distinct types of spermatocytes readily divisible on the basis of size. Those characterized by the larger size are about twice the average diameter of the smaller ones and vary from them in behavior in the two maturation divisions. But this variation in behavior concerns the achromatic structures of the cell and seems to be due to the much greater amount of cytoplasmic and archoplasmic material present in the larger cells. It is extremely interesting to note that as regards the behavior of the chromosomes these two sizes of spermatocytes are essentially identical. Indeed, the chromosome groups of the small cells differ in no respect from those of the large type. The elements are no smaller than in many of the large cells and present the same constancy in form and the same size relations characteristic of the large type of cells. Figs. 16, 17, 18 represent the chromosome groups of the small type of spermatocytes. Many more were carefully studied and drawn and all show the same characteristic shapes and size relations typical of the spermatocyte chromosomes. In fact, the only reason more of these were not used is that they are not so desirable for study owing to the difficulty in drawing them due to their closer crowding in the metaphase.

The shape of the daughter chromosomes as they move apart to the poles in the anaphase of the first spermatocyte mitosis is quite characteristically different for the different types of chromosomes (Figs. I, II, IV, *h*, III, *i*). Those resulting from the division of cross-shaped tetrads have the three lobed appearance shown in Fig. I, *h*. The daughter chromosomes resulting from the division of the double-V-shaped tetrads have the shape shown in Fig. II, *h*, and are essentially single-V-shaped chromosomes, as is shown at a later stage. Those resulting from the division of the double-rod tetrads, as they move toward the poles, have the form of single rods, slightly constricted near the middle. I have been unable to identify positively the division products of the tetrad which undergoes its reduction division in the first mitosis, but in several anaphases six of the daughter chromosomes of each group are V-shaped, and it is probable that the sixth one of this shape is the chromosome in question. This is rendered more certain by the observation that in these cells there are but

four of the rod-shaped chromosomes exclusive of the accessory, which, of course, is present in but one of the chromosome groups.

The chromosome groups, as seen in the metaphase of the two cells derived from one primary spermatocyte are shown in Plate II., Figs. 19, 20. The most striking fact to be observed is the absence of the accessory chromosome in one of the cells. Even a superficial examination, however, shows that in shape and relation to the mantle fibers the chromosomes are of several different types, and that these characteristics coincide with what would be expected from a study of their earlier history. The chromosomes derived from the cross-shaped tetrads have altered their shape considerably since last seen in the anaphase of the first maturation division. They are much shorter and thicker and are now bilobed bodies—the constriction between the lobes representing the plane of transverse cleavage. The attachment of the mantle fibers seems to be at no particular point but may be at any part of each flattened end of the dyad.

The structural peculiarities of the chromosomes derived from the double-V-shaped tetrads are much more characteristic. In shape the dyads of this type resemble those just described to some extent, but, except in size, bear a more striking resemblance to the double-V-shaped chromosomes from which they are derived. One end, usually the one nearest the center of the equatorial plate, is broader than the other, and the entire structure very evidently corresponds to the single-V-shaped chromosomes of the first spermatocyte anaphase. The mantle fibers are always attached to the broader end of the dyad, this fact being even more characteristic than the shape. The appearance of these chromosomes while in the act of division might lead one to believe that the resulting division is a longitudinal one, but such a conclusion would ignore entirely the previous history of this type of chromosomes during the prophase and metaphase of the first maturation division.

Chromosomes L, M, O and P, in the second spermatocyte (Figs. 19, 20) are the product of the longitudinal division of the rod-shaped tetrads. They are dumbbell-shaped dyads with a mantle fiber attached to each end. The constriction at the middle of each represents the plane of transverse division. Chro-

mosome N shows a considerably different shape, corresponding to its different history. It is shorter and in one plane broader than the other dyads derived from the double-rod-shaped tetrads of the first spermatocyte. It has been already shown that chromosome N of the first spermatocyte probably undergoes a transverse division while the other tetrads are dividing longitudinally, and we would, therefore, expect the products of this division to present a different appearance from the other dyads. As a matter of fact, it is of quite a different shape from the others derived from the double-rod tetrads.

The differences in size between the various chromosomes of the different types is, of course, only half as great in the second spermatocyte, as it is in the first spermatocyte, and therefore there is not such certainty in identifying the various individuals of the different types. But the same size ratio seems to exist and the chromosomes of the different types can readily be arranged in a graded series as regards size, just as in the first spermatocyte.

It has been shown by this study that the chromosomes of *Scolopendra heros* cannot be considered as ephemeral structures, which have one appearance in one cell and present an entirely different form in another cell of similar history. Any study except a very superficial one must lead to an entirely different conclusion. By a study of many hundreds of cells in various stages of mitosis it has been found that the number of chromosomes in the primary spermatocytes is absolutely constant and invariable. Furthermore, these chromosomes show other characteristics, which speak very strongly for their individuality. The ordinary chromosomes are divisible into three types on the basis of the shape they assume in the prophase and metaphase of the first maturation division, and in their relation to the mantle fibers of the spindle. The individuals of each type of structure are invariably of the same number and in all favorable cases each chromosome of a given type is distinguishable from the others of a similar shape by a difference in size.

In addition, several of the chromosomes possess certain individual peculiarities aside from shape and size, which serve

further to characterize them. One of the cross-shaped tetrads is tardy in orienting itself in the plane of the spindle. Another chromosome, one of the rod-shaped ones, shows a much more striking and fundamental peculiarity, in that it differs from all of the rest of the bivalent chromosomes in the plane of its divisions in the first and second spermatocytic mitoses. The accessory chromosome shows still more striking peculiarities, differing from the others in its origin, valence, behavior in the prophase, relation to the mantle fibers of the spindle and in its distribution to but one half of the resulting cells.

All of the facts enumerated above offer evidence which seems conclusive that the chromosomes of *Scolopendra heros*, during the spermatocyte stages at least, must be considered as distinct entities, each one possessing certain well defined peculiarities which are as characteristic for any given chromosome of the spermatocytes as are the peculiarities of a species of animals. I believe that eventually in many animals it will be possible to make this statement still broader and to demonstrate the continuity of the individual elements from cell generation to cell generation. We will, then, be able to say that, while in different cell generations or different conditions of cell activity, the appearance and behavior of any given chromosome may be quite different, just as is true of many animals in different stages of their existence, yet in a similar cell generation any particular chromosome will present the same appearance and will behave in the same manner. I believe that the condition described above is true in *Scolopendra heros* but several facts conspire to make it impossible of physical demonstration.

These difficulties are mechanical difficulties and have to do with the small size of the chromosomes in the spermatogonial stages and the close aggregation of these elements in the karyosphere of the growth period. The difficulties due to the small size of the chromosomes in the spermatogonial stages appears insurmountable, and the only evidences of individuality which they present have to do with their absolute constancy in number, and with the very characteristic behavior of the accessory—it being the only element which can be identified at all stages. We might reason from this that because one of the elements

displays unmistakable individuality all of the chromosomes possess individuality. This argument has been made in other cases and, while the continuity of the accessory chromosome *does* offer valuable evidence in support of the individual continuity of the chromosomes in general, it cannot be said to establish the truth of the general theory.

The difficulty of establishing the individuality of the chromosomes during the growth period would seem fully as great as during the spermatogonial period. During all the stages in which the karyosphere exists the chromosomes are so densely aggregated that it is impossible to distinguish the separate elements. But even at this time it is possible in favorable cases to distinguish the accessory chromosomes and to discern the outlines of some of the other elements. Furthermore, as I have shown in previous papers (Blackman, :05, *op. cit.*) the chromosomes enter the karyosphere as distinct bivalent elements, and at the end of the growth period arise from it as distinct chromatic segments of the same number and character as in the earlier stage.

The chromatin segments entering the karyosphere are bivalent threads formed by the union and subsequent diffusion of two spermatogonial chromosomes. The point of union of synapsis shows very plainly as a distinct interruption of the chromatin granules near the middle of the segment, the interval being bridged by linin fibers. In favorable sections of the karyosphere (*i. e.*, those in which the stain has been sufficiently extracted) it is seen that this body is made up of a number of chromatin segments closely massed about the accessory. The chromosomes on leaving the karyosphere are of the same structure as when they entered, are of the same number and in appearance differ from those of an earlier period in size only. In fact, the larger spermatocyte chromosomes possess nearly as great a bulk as the entire chromosome group of the spermatogonium, this immense increase in size being accompanied by a growth of other parts of the cell, which is proportionally even greater.

It would appear then, that during certain stages of the spermatogenesis of *Scolopendra* it is possible to demonstrate absolutely that each chromosome is a distinct unit characterized by certain definite and constant peculiarities and that the continuity of

each element can be traced from the early prophase of the first spermatocyte to the anaphase of the second maturation division. In other words, it is evident that during this very important period of their history the chromosomes show complete individuality. In other stages, namely, in the spermatogonia and during the growth period, it cannot be claimed that the continuity of the chromosomes is actually demonstrated in *Scolopendra*, although evidence strongly supporting such a view undoubtedly exists.

Summary.

The chromosome group of the primary spermatocytes of *Scolopendra heros* is made up of sixteen bivalent chromosomes (tetrads) and one univalent chromosome (dyad), the accessory chromosome.

The chromosomes show such constancy in shape in the prophase and metaphase of the primary spermatocytes, and in their relation to the mantle fibers of the first maturation spindle, that they seem naturally to group themselves under four distinct types. These may be designated respectively, as the cross-shaped tetrads, the double-V-shaped tetrads, the rod-shaped tetrads, and a single-rod-shaped dyad.

The cross-shaped tetrads are six in number and may be arranged in a graded series as regards size, the difference in bulk being sufficiently great to allow the individual chromosomes of this type to be distinguished. One of the chromosomes of this type (the largest one) can furthermore often be identified by its tendency to lag behind the others during the early metaphase.

Five of the tetrads are of the double-V shape. The individuals of this type also may be distinguished by differences in bulk.

The rod-shaped tetrads are present to the number of five. These show constant size relations and may readily be arranged in a graded series as regards magnitude. One of the tetrads of this type differs from the others in the form it assumes during actual division. It seems to divide transversely, while the others are dividing longitudinally.

The accessory chromosome is univalent and passes to one of the secondary spermatocytes without division. During the

metaphase it is connected by mantle fibers to only one pole of the spindle.

As a result of the first spermatocyte mitosis fifteen of the tetrads are divided longitudinally (equationally), while the one remaining tetrad divides transversely (reductionally). The failure of the accessory chromosome to divide is, also, in effect a reductional division.

During the later stages of the first maturation division and during the metaphase of the second spermatocyte, it is possible to distinguish the daughter chromosomes derived from the several types of tetrads, by their shape and their relations to the mantle fibers. The individuals of the various types show the same size ratio as exists between the chromosomes of the first spermatocyte, although, of course, the actual difference in bulk is but half as great.

The above results seem to establish as a fact, or at least as a very strong probability, that the chromosomes of *Scolopendra heros* are distinct and definite individuals, which, under similar circumstances, *i. e.*, in the same cell generation, show a remarkable constancy in form, relative size, and in their attachment to the mantle fibers. This constancy of form, size and behavior, affords a strong argument in favor of the theory of the individuality of the chromosomes in this species in particular and adds support to the evidence derived from the study of other forms, to the general application of the theory.

Laboratory of Zoölogy,
Syracuse University,
April 11, 1910.

Explanation of Plate I.

All drawings were made by the author with the aid of a camera lucida. The optical equipment consisted of a Zeiss apochromatic objective of 2 mm. focus and a number 12 compensating ocular, the source of the light being a Welsbach mantle The original magnification was 2,300 diameters and the drawings were reduced one fifth in reproduction, making the final magnification 1,840 diameters.

The seventeen chromosomes arranged in each horizontal row represent the chromosomes of a single cell, as seen in a side view of the spindle in the metaphase. They are arranged as follows: the six which show the characteristic cruciform shape comprise the first six of each row and are lettered A, B, C, D, E and F. Those showing the double-V shape—five in number—are lettered G, H, I, J and K. Those corresponding to the double-rod type of structure are lettered L, M, N, O and P. The seventeenth and last chromosome in each row is the accessory and is distinguished by the letter Q. The individuals of each type of structure are further arranged in a graded series as regards size, the largest first, etc.

Figs. 1–15 represent the chromosome groups in the metaphase of the large type of first spermatocytes.


A B C D E F G H I J K L M N O P Q
1
2
3
4
5
6
7
8
9
10

Explanation of Plate II.

Figs. 16–18 represent the chromosomes of the small type of spermatocyte in a similar stage.

Figs. 19 and 20 represent the chromosomes of the two second spermatocytes, derived from one primary spermatocyte. The individual chromosomes are arranged so as to correspond in position to the parent chromosomes as seen in the other figures. As will be seen, in one cell the accessory chromosome is not present, it being distributed to only half of the secondary spermatocytes.


A B C D E F G H I J K L M N O P Q
11
12
13
14
15
16
17
18
19
20

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BIOLOGICAL BULLETIN

MANGANESE OF THE LAMELLIBRANCHS.

H. C. BRADLEY.

Department of Physiology, University of Wisconsin, Madison, Wis.

In 1892 Griffiths published an account of the finding of manganese in the blood of the lamellibranch, *Pinna squamosa*.[1] So far as I am aware this result has never been confirmed nor until recently has an examination of other molluscs led to an extension of this isolated fact. To the student of comparative physiology such a finding must be of considerable interest, adding one more respiratory mechanism to the list of five or six with which we are familiar. At the same time it is highly improbable that *Pinna squamosa* is the only mollusc utilizing manganese in a respiratory compound; we should expect to find the element in other forms more or less closely related to it. It is a matter of common observation that the respiratory proteins of the more highly organized animals fall into a few general types, such as haemoglobin or haemocyanin, and that while individual bloods may show subtle biological differences within one of these groups, there is never any difficulty in determining chemically whether a blood pigment is a haemocyanin, a haemoglobin, or some other typical complex. The effective respiratory mechanisms are thus quite limited, so that we do not expect to find a single member of a family possessed of a blood protein totally unlike the other members of that family.

For this reason we have extended the investigation of this point somewhat with a view to determining what other lamellibranchs are provided with a respiratory mechanism similar to that of *Pinna squamosa*. The most notable group which we

[1]Griffiths, *Compt. rend. de l'Acad. des Sci.*, CXIV., p. 840.

have thus far found to utilize the element manganese is the Unionidæ, the common fresh-water mussels. Since 1906 when the element was first noticed in the specimens obtained from the Madison Lakes of Wisconsin,[1] we have examined many hundreds of specimens from the Mississippi basin, St. Lawrence and Atlantic coast drainage. In not a single specimen has the element been wanting or small in amount. It is obvious that a single individual which failed to show manganese, or contained only a trace of it would be sufficient to cast grave doubts upon the normality of the element and lead one to ascribe an adventitious character to it. But in every case manganese has been abundant. The reactions for its identification are fortunately brilliant and decisive and at the same time indicate very well the relative amount of the element. The quantitative determinations show that the metal occurs in quite uniform amounts in the various specimens examined.

To summarize briefly the results: Some twenty-four analyses were made quantitatively upon material from about Madison. Some of these analyses were made upon single specimens of *Anadonta* or *Unio*, more were made upon a sample taken from the dried and pulverized tissues from a large number of specimens secured at one time from a given locality. Many of these analyses therefore represent the average of fifty or a hundred individuals. The average of the twenty-four analyses shows 21.8 per cent. of ash in the tissues, 4.52 per cent. manganese present in the ash and 0.95 per cent. manganese in the tissues. Mussels from the Wisconsin River averaged about 14.5 per cent. ash, 2.4 per cent. manganese in the ash and 0.35 per cent. in the tissues. From the Temagami Reserve of Ontario mussels averaged 15.4 per cent. ash, 3.1 per cent. manganese in the ash and 0.45 per cent. in the tissues. Specimens obtained from a great number of localities in Michigan, Illinois, Wisconsin, Indiana and Iowa average about 17.0 per cent. ash, 3.4 per cent. manganese in the ash and 0.60 per cent. in the dry tissue. A number of normal, average sized specimens from Lake Mendota were dissected into their more prominent tissues or organs. Analyses of these fractions gave the following results:

[1] Bradley, *Jr. Biological Chem.*, III., 151, 1907.

Tissue.	Per Cent. Ash.	Per Cent. Mn in Ash.	Per Cent. Mn in Tissue.
Muscle	6.0	4.87	0.293
Stomach; fibrous part	14.5	5.73	0.831
Stomach; non-fibrous part	32.0	4.69	1.492
Nephridial organs	27.0	5.31	1.434
Gills	33.5	4.89	1.638
Mantle	48.0	5.12	2.457
Liver	39.0	5.85	2.107
Eggs	37.0	2.024	0.749

Perhaps the most interesting result of these analyses was the presence of the element in the eggs and embryos, showing clearly that manganese is not an adventitious element picked up by the adult and held in the tissues from inability to excrete it—as, for example, iron compounds may be in the mammalian spleen after certain diseases involving great destruction of red corpuscles.

Another interesting point brought out in the above table is the exceedingly high mineral content of such a tissue as the mantle. Its ash content of 48.0 per cent. puts it in a class with mammalian osseous tissue, though unlike the latter the mantle is soft and pliable. This is a type of tissue resembling no vertebrate organ or tissue of which we are aware. It seems probable that its function as a gland secreting the shell must have some connection with the high mineral content.

Having established as we believe the normality of the element manganese in the tissues of the Unionidæ, the question as to its origin naturally presents itself. It is hardly to be supposed that an animal of so great complexity as a lamellibranch would actually concentrate the element from its highly dilute solutions in lakes and streams. Such concentration is usually performed by lower forms of life which are then obtained as food. In the case of the Unionidæ the food origin of manganese is much more clearly apparent than is the food origin of copper in many of the hæmocyanin-bearing animals. The waters in which the mussels are found have invariably contained the brown masses of the manganiferous crenothrix mixed with diatoms, and other plancton forms which very probably contain manganese also. And this brown slime seems to be the normal food of these mussels so far as our observations extend. In Ontario

there are many lakes set in clean rocky basins, and fed by streams which leave little or no manganese stain on the rocks, and which appear to be free from the masses of crenothrix. In such lakes we have been unable to find mussels. In other lakes in the same region where seepage through glacial drift was apparent, or where the tributary streams flowed through such drift, discoloration of the stones, evidence of the presence of crenothrix, and the presence of the mussels seemed always to go together. For example in the Temagami Reserve, Lake Temagami itself is characterized by its clear water, and its clean rock basin. In certain parts occur limited areas of drift—sand and gravel—which are of insignificant amount. But though the bottom afforded, where the lake washed such drift areas, looked promising no mussels were found and the sandy stretches were apparently free from crenothrix. To the north of Lake Temagami are several lakes which lie in basins of glacial drift. In Sucker Gut, for example, sand and gravel beaches are abundant, the tributary stream flows through many miles of drift and carries enough manganese and iron in solution to stain its stones and pebbles strongly brown and black. The sand itself is stained with iron, and the brown slimy masses of crenothrix are abundant. In this lake and its tributary stream we found enormous numbers of small mussels wedged in thickly between pebbles or projecting from the sandy bottom. The many obvious examples in this region of the simultaneous presence of manganese, crenothrix and mussels, or of the absence of all three is probably more than a mere coincidence. We believe that more careful examination would show that the mussels require such manganiferous food as crenothrix and that they cannot live in waters where such food does not thrive.

In growing the mussels in aquaria the specimens always carry enough of the manganiferous organisms clinging to them so that in a few days an abundant development of the bacteria results. In this way several hundred grams of the dry organisms have been obtained for analysis. Such specimens are mixtures of a great variety of organisms and thus show large differences in chemical content. The ash content of such plancton crops vary from 24 to 76 per cent. of the dry weight; the manganese

from 0.13 to 1.84 per cent. of the dry weight. It has thus been possible to obtain, through the agency of these organisms, several grams of manganese from running water which contained about 0.0000066 per cent. of that element. The concentrating efficiency of these lower forms is therefore of a high order.

In secreting the shell, the Unionidæ deposit salts of manganese as well as of calcium and magnesium. The nacer of the shells, carefully freed from contaminating material, always gives a strong reaction for manganese; its presence in the shell is as characteristic as its presence in any of the tissues. It was thought therefore that an examination of fossil shells of the Unionidæ would be of interest in determining whether the manganese is of comparatively recent occurrence in these animals or whether it is a metabolic characteristic of long standing. So far we have had the opportunity to examine but one well preserved fossil shell. This was a specimen obtained through the courtesy of Dr. George Wagner who published a description of it in *Nautilus*, Vol. 18. The nacer of this shell was perfectly preserved, retaining its luster, though friable and crumbling to a powder easily. The fossil nacer gave 0.085 per cent. of manganese, while fresh shells of the present period frequently contain as much as 0.148 per cent. Thus it can be definitely asserted that the Unionidæ in pre-Pliocene times were using the element manganese as we find them today. It seems probable that the marine ancestors of the Unionidæ were themselves manganiferous. The fact that at least one marine lamellibranch is known makes such an assumption the more plausible.

To determine whether other marine lamellibranchs utilize manganese in this same way, an examination of the common forms along the coast of southern Massachusetts was made at the Woods Hole laboratory. In several forms the elements could usually be detected as a trace, but in such cases no import can be attached to its presence except as indicating that there is some marine low form of life serving as food for lamellibranchs, which also carries manganese. In *Pecten* the manganese was variable, sometimes large in amount, at others very small. It was frequently found abundant in the stomach contents. In *Modiola modiolus* the element was present in every specimen

examined, and it seemed to be rather uniform in amount. It was present in every tissue, and in the nephridial organs it was really abundant. In most of the tissues it was not at all comparable to the amount present in the Unionidæ, approximately 0.1 per cent. or less of the dry material. It will be remembered that the nephridial organs of *Modiola modiolus* are usually pigmented a dark brown—in all of some fifty specimens examined by us this brown pigmentation was prominent. It is possible that this lamellibranch deposits manganese obtained with its food in the nephridia in an attempt to excrete the element; that it is in this case adventitious and analogous to deposits of iron-containing pigment in mammalian tissues as the result of pathological conditions. It is interesting to note however that this marine mussel which stands morphologically fairly close to the Unionidæ, should appear to utilize the element so characteristic of the latter family.

It is our belief that other lamellibranchs will be found which, like *Pinna squamosa*, the Unionidæ and perhaps *Modiola modiolus*, utilize the element manganese in their metabolic processes. Such a chemical relationship may be useful in suggesting the lines of the evolutionary process which has led to the development of the present forms. It is our expectation to continue this line of investigation as opportunity permits.

THE SPERMATOGENESIS OF EUCHROMA GIGANTEA.

M. LOUISE NICHOLS.

The largest of the buprestid beetles, *Euchroma gigantea*, is native to Central and South America and is commonly found sunning itself on the trunks of trees. In such situations the beetles are not difficult to capture, as their movements are rather sluggish until they become thoroughly alarmed. The specimens from which the present study was made were taken at Culebra, Panama, in the month of August, at which time some of the beetles were mating, the male apparently attracting the female by a clicking sound produced by the elytra.

Upon sectioning the testes, I was surprised to find a complete series of stages from the spermatogonia to the mature spermatozoa, the younger stages not being confined to the larvæ or pupæ, as is frequently the case in insects. The testes were fixed in Gilson's mercuro-acetic-nitric solution or in Fleming's strong solution and stained with iron-hæmatoxylin or with saffranin and malachite green.

In the development of the germ cells of insects, as is well known through the results of the researches of Montgomery, Wilson, Stevens and others, there are present chromosomes which have been called heterochromosomes or idiochromosomes. Wilson (1909) has shown for the Hemiptera that in certain forms the idiochromosomes are equally well developed in both sexes, in others the male possesses one well developed, the other reduced in size, while in still others one is entirely lacking in the male. Stevens (1906) found somewhat similar conditions in the Coleoptera. Thus, the Elateridæ and Lampyridæ possess only the odd chromosomes, while the families Chrysomelidæ, Coccinelidæ, Scarabidæ, Silphidæ and Buprestidæ show one of the idiochromosomes reduced in size. In Carabidæ some members have an unequal pair of heterochromosomes, others an odd chromosome. *Euchroma gigantea*, as a member of the family Buprestidæ, belongs in the second of these groups (Figs. 21–22).

Besides the idiochromosomes, Wilson discovered in the Hemiptera a pair of chromosomes equal in size but noticeably smaller than the others, which he designated as *m*-chromosomes. According to the researches of Stevens these are occasionally present in the Coleoptera, *i. e.*, in *Trirhabda virgata* and *T. canadense* and in an unidentified buprestid. They likewise are represented in *Euchroma* (Figs. 19, 23, 24). In addition there are, in the spermatocytes, eleven chromosomes of more nearly equal size, making the total reduced number thirteen.

In most forms heretofore studied, the idiochromosomes are evident not only at the time of mitosis but also in the resting stage and prophases, for while the other chromosomes become resolved into the nuclear network, the idiochromosomes remain compact. It is in the manner of formation of the chromosomes during the prophases of the first maturation division and in the fact that neither at that time nor in the previous stages are the idiochromosomes distinctly different in behavior from the other chromosomes that the chief interest of the spermatogenesis of this beetle lies.

The nuclear network of the last generation of spermatogonia is of delicate texture. Chromatin masses occur at intervals, at first few in number and without constancy of position or shape (Fig. 5). The masses gradually become more distinct and form elongated threads near the center of the nucleus (Figs. 6, 7). The network breaks away from the nuclear wall and the synapsis is inaugurated (Figs. 8, 9). During this time there is no evidence of the idiochromosomes being isolated from the synaptic threads or failing to take part in their formation, nor, in the resting spermatocyte, do the idiochromosomes differ from the others. Stevens (1906) has reported a somewhat similar condition in the beetle *Tenebrio molitor*.

The nuclear network of the resting spermatocyte is more clearly defined than that of the spermatogonia and bears chromatin masses distributed with a fair degree of regularity (Fig. 10). This condition, however, does not continue. Instead of the usual spireme formation, the chromatin granules commence to migrate towards a specialized area within the nucleus (Figs. 11–14). The final result of this process is the formation of a

dense mass of chromatin in one part of the nucleus, the remainder of the nucleus being occupied by a fine network. It sometimes happens that more than one of these areas of aggregation develops (Figs. 15–18).

The outlines of the separate chromosomes may be seen, although they lie very close together. The nuclear network next begins to show a parallel arrangement of threads preparatory to the formation of the spindle (Figs. 17–18).

As is the case with other members of this order, the small heterochromosome is separated from the larger by the first division (Fig. 21). In mitosis the *m*-chromosomes tend to divide somewhat later than the others (Figs. 23–25).

After the second division the spindle fibers persist in the cytoplasm. They gradually cease to run parallel to each other, become more or less interlaced, and finally are arranged in spiral form (Figs. 26–28). Later they are converted into the tail of the spermatozoön (Fig. 32).

The chromatin of the spermatid at first condenses in large measure at the side of the nucleus nearest the spindle fibers, but as the latter lose their regularity of arrangement, the chromatin is dispersed throughout the network (Figs. 26–27). It next breaks into small fragments which migrate to the center of the nucleus, one mass, however, (probably the heterochromosome), remaining distinct from the rest (Figs. 29–32). As the nucleus approaches maturity, it elongates and the chromatin becomes finely granular, although fragments in chain form are still distinguishable (Fig. 33).

Discussion of Results.

A number of workers (see Blackman, 1903) have described "chromatin nucleoli." They occur in a wide variety of plant and animal groups and usually during a period of growth. In oögenesis and spermatogenesis they may be present in the oö- and spermatogonia as well as in the later stages. Among the groups in which they have been discovered are echinoderms, molluscs, arthropods, amphibians and mammalians, also in protozoa and plants. It may prove instructive to compare some of the more striking of these cases with that of *Euchroma*.

Sometimes the karyosphere is formed immediately after synapsis. Illustrations of this are furnished by the oögenesis of the dragonfly, *Plathemis* (McGill, 1906), and the pollen development of *Sarracenia* (Nichols, 1908). In this plant the karyosome is formed from the synaptic threads through an absorption of the chromatin substance by a nucleolus (Plate III., Fig. 1). The achromatic substance (linin) remains as a dense mass after the chromatin has passed by drops into the closely contiguous nucleolus. There is, therefore, a rather conspicuous separation here of chromatin and linin.[1]

In *Plathemis* the synaptic threads gather closely around the nucleolus and form what Miss McGill calls a double nucleolus (oxyphil+basophil). There is plainly an interchange of material between the two parts of the nucleolus and, as the network again expands, masses of basophil substance are elaborated within the nucleolus and pass out on to the network.

In *Euchroma* the transfer of chromatic material to the karyosome is less direct, inasmuch as the synaptic threads are first extended into the network of the resting spermatocyte (Fig. 10; Plate III, Fig. 4, *a*). The latter possesses no large nucleolus such as is present in the plant nucleus. Nevertheless a center of activity arises, towards which the chromatin passes and simultaneously also the linin, at first in the form of streamers radiating from the karyosphere (Figs. 11–12). Later, as the chromosomes become distinct within the karyosphere, the linin is incorporated with them (Figs. 16–18).

Blackman (1903) interprets the karyosphere of the spermatocytic prophase of myriapods as a mass of fine, granular filaments closely gathered about the accessory chromosome. As the nucleus approaches mitosis, the threads emerge from the karosphere, shorten and thicken to become the mitotic chromosomes (Plate III, Fig. 3, *a* and *b*). There is here no separation of chromatic from achromatic substance, only a strong attraction for both to a definite region of the nucleus. As compared with *Euchroma* this tendency manifests itself much earlier, for the karyosphere has already begun to resolve itself into definite chromosomes in

[1] A closely similar behavior of chromatin has been described for *Spirogyra* by Berghs (1906).

Scolopendra at a period when in *Euchroma* it is condensing (Plate III., Figs. 3 and 4). In *Scolopendra* there is likewise a karyosphere present in the spermatogonia, whereas it is lacking in *Euchroma*.

A somewhat different condition is described by Eisen (1900) for *Batrachoseps*. A karyosphere (chromoplast) is present in this object. At first it lies free in a vacuole, but later appears to attract "leaders" which might be compared to the radiating rays of *Euchroma* (Figs. 11–12).

Through these leaders small particles of chromatin are projected into the karyosphere and again emerge from it. The convergence of the leaders towards the karyosphere apparently corresponds to a synapsis stage, and as they finally break apart each chromosomal thread receives a portion of the karyosphere which is gradually distributed through the length of the thread[1] (Plate III., Figs. 2, *b* and *c*).

The condition in echinoderm eggs treated with Mg salts (Wilson, 1901) and in normal mouse eggs (Sobotta, 1895) is in some degree similar to that of *Euchroma*, for although there is apparently no separation of linin and chromatin, the karyosphere breaks up directly into the mitotic chromosomes (Plate III., Figs. 5, *a* and *b*).

What bearing have these facts on the question of the individuality of the chromosomes? In the paper on *Sarracenia* I suggested that the phenomena there "might be explained on the assumption that the morphological basis of the chromosomes remains in the linin while that portion of their substance which causes them to color deeply is absorbed by the nucleolus. If a similar interpretation be applied to the case of *Batrachoseps*, it will be seen that the linin retains its individuality more clearly than the chromatin, which may be transferred to the karyosphere. In the myriapods there is apparently no separation of chromatin and linin, but a tight coil of threads, consisting of

[1]Janssens (1905)in his later work on *Batrachoseps*, remarks: "il nous semblait très probable que les chromoplastes resultaient de la soudure très intime des chromosomes aux telophases à la faveur de rapprochement." "Il semble qu'une substance intensement siderophile, une sorte de nucleine, soit venue empâter tout le pole de la figure centre à ce moment. Il se peut qu'il s'agisse là d'un exsudat des chromosomes eux-mêmes."

both, forms the karyosphere. Possibly this might be regarded as a continuation and exaggeration of the synaptic condition, and, if this were true, a series of consecutive stages might be conceived between the typical synapsis and post-synapsis of most animals and plants and the extreme conditions presented by *Sarracenia*.

In the echinoderms, in *Mus* and in *Euchroma* a similar tendency to condense reappears and in the germ cells later than synapsis, owing, no doubt, to a chemical condition of the nucleus varying from the usual type. Here there is in one case (*Euchroma*) a more rapid condensation of chromatin than of linin and a consequent partial separation, while in the other (*Mus*, echinoderms) no such separation is apparent, chromatin and linin condensing simultaneously (Plate III., Figs. 4 and 5).

There is, however, little reason to believe that the difference in the method of formation of the spermatocytic chromosomes of *Euchroma* and most others insects is fundamental. The gradual change in coloration as resting chromatin becomes active goes to show that there is a chemical change in progress from less acid to more acid condition, accompanied by a condensation of substance. According to the differing constitution of different nuclei, this chemical activity might be confined to one center or distributed through the nucleus around several centers. If the latter were the case, the network of resting spermatocytes would break at various points and, condensing, form the chromosomes, but if there were but one center, the condensation would occur within a more circumscribed area. In *Euchroma*, while the latter method is more common, it may happen that the centers of condensation are multiplied (Fig. 15).

An interesting question concerning the relation of the chemistry of the nucleus to the individuality of the chromosomes presents itself at this point. If it be true that the aggregation of chromatin is accompanied by a decomposition of nucleo-proteids and a reduction of chromatin to nucleic acid or a simple compound of that acid and also true that the chromatin may be separated from the linin and gathered into a karyosphere, may it not be possible that the linin network is not homogeneous as regards its chemical character, but that in different areas are

developed different proteid substances which, when combined with the nucleic acid of the karyosphere, become active and colorable by chromatin stains. It may be that usually in the development of the germ cells the nucleic acid becomes chemically dissociated, but not visibly separated from these proteid substances, and consequently no karyosphere is present, simply chromosomes consisting of a condensed linin framework surrounded by nucleic acid.

PHILADELPHIA NORMAL SCHOOL,
June 4, 1910.

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Explanation of Plate I.

The drawings were made with the camera lucida, Zeiss microscope, oc. 12, obj. oil immersion 1/12. They have been enlarged to twice the diameter and reduced one half.

Figs. 1–4. Spermatogonia. Prophases.

Figs. 5–7. Last generation of spermatogonia. Prophases.

Figs. 8 9. Synapsis.

Fig. 10. Resting spermatocyte.

Figs. 11 18. Spermatocytes. Prophases of the first maturation division.

Figs. 19–20. Equatorial plates of the first maturation division. 13 chromosomes.

Figs. 21. The heterochromosome x.

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Explanation of Plate II.

Fig. 22. The heterochromosome x.

Fig. 23. Side view of the first maturation division. Metaphase.

Fig. 24. Anaphase. Late division of the microchromosomes.

Fig. 25. Telophase. Traces of the late division of the microchromosomes.

Figs. 26–33. Spermatids.

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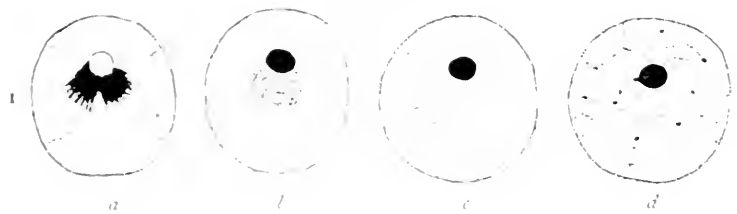
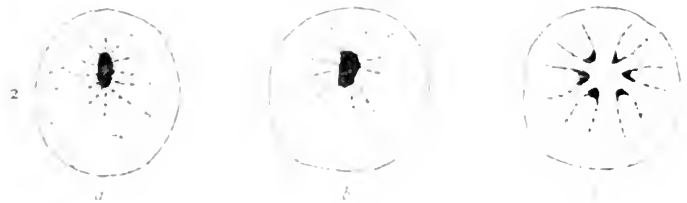
Explanation of Plate III.

Figs. 1–5. Diagrams showing the relation of the karyosphere to chromatin and linin.

Sarracenia.



Isatis tinctoria.



Silene nivea.

Fumaria.

Mus. Echinoderm.

M. LOUISE NICHOLS.

ON THE NATURE OF THE CANALICULAR APPARATUS OF ANIMAL CELLS.[1]

R. R. BENSLEY.

During the twelve years that have elapsed since the discovery by Golgi of the internal reticular apparatus of the nerve cell, much attention has been devoted by investigators to the consideration of similar reticular structures in cells. Our knowledge has progressed along several different lines, which have been determined more or less by the techniques employed by different investigators, and although all are not agreed as to the identity of the structures so revealed, their consideration together seems to be justified by the great similarity in configuration and location which these elements possess.

It is not necessary in this paper to review in extenso the literature on this topic, for that is well covered by the summary given by Holmgren ('02) in Merkel und Bonnet, Ergebnisse, etc., in which also the different theories of the nature of these structures are well brought out. The more recent contributions are considered in the article of von Bergen ('04) and that of Legendre ('08 '09). It will suffice here to summarize the progress that has been made along the different lines of investigation, and to consider the interpretations of these structures which have been advanced by different workers.

Golgi ('98) first described the internal reticular apparatus in the cells of Purkinje of the cerebellar cortex, where he demonstrated it by means of a modification of his well-known chrome silver impregnation method. He describes it as a closed net of fine fibers occupying the intermediate zone of the cell protoplasm and separated by a distinct interval from the nucleus on the one hand and from the surface of the cell on the other, that is to say, there was a zone of protoplasm on the periphery of the cell which was wholly free from the fibers constituting the network. Toward the nucleus the net sent out fine fibers so that the perinuclear space was not wholly devoid of fibers.

[1]From the Hull Laboratory of Anatomy, University of Chicago.

In his later studies Golgi confirmed this result for other types of nerve cells including spinal ganglion cells, spinal cord cells and cells of the cerebral cortex. In some of these cases he found the network provided with freely ending branches which terminated in a small swelling. In some cases, too, the fibers of which the network was composed had varicose swellings on them, and nodal enlargements, and, in some cells, he even found two concentric nets which differed inter se by the amplitude of the meshes and the size of the fibers. Golgi in all of his papers expressed himself with great reserve as to the nature of the network, but was confident that they had nothing to do with the neurofibrils and that they were not canals which had been filled with the silver precipitate. He was moreover certain that they were entirely intracellular and that they had no communication with extracellular structures.

In the meantime Golgi himself and his students had been extending the field of investigation to other than nervous tissues, and it had developed from these investigations that the reticular apparatus was not confined by any means to nerve cells but was present in a large variety of cells from different sources. For example, Negri ('00) demonstrated an apparatus of this sort in the cells of the pancreas and of the parotid gland of the cat, and in the thyroid epithelium of the dog. In these cells the situation of the network was quite characteristic and recalled the observations of Golgi that in young nerve cells with excentric nucleus the reticular apparatus was also excentric and located for the most part at one pole of the nucleus. In the epithelial cells, namely, it was found that the reticular apparatus was located near the nucleus, but between the latter and the free border on the lumen or surface, that is, it was distal to the blood vessels. Later, similar nets were found in the cells of the epididymus by Negri, in cartilage cells by Pensa ('01), and in striated muscle fibers by Veratti ('02).

The observations of Golgi were confirmed by a number of observers, using this method or one of the silver reduction methods of Cajal. Retzius, for example, obtained good impregnations of the apparatus in the nerve cells of the cat and rabbit, which corresponded in their salient characters with those of Golgi but

were not nearly so complete judging by the figures published. In view of this it is surprising to note that Retzius ('00) found, in some of the cells, the fibers of the network communicating by a branch with the surface of the cell, which Golgi had never observed in his more perfect preparations.

In a recent publication Golgi has adopted a new method for the demonstration of the reticulum which is based on the silver reduction method of Cajal. In view of this fact it is proper to mention here that Cajal ('07) has also studied the reticular apparatus in the nerve cell, which he accepts, contrary to Golgi, as a tubular apparatus to which he applies the name "Conduits de Golgi-Holmgren," thus accepting the interpretation of Holmgren that they are the same as the so-called juice canals described by him. He regards the appearances seen in his preparations as due to the presence of canals filled with a coagulable substance which has an affinity for colloidal silver. He also notes differences in the behavior of the apparatus in different animals, from which he concludes that the contents of the canals in different cells may have different chemical properties.

By the same methods as those employed by Cajal, Sanchez demonstrated an exceedingly interesting system in the striated muscle fibers of mammals and insects. In the former this system did not communicate with structures outside of the cell but sent free ending branches which terminated just under the sarcolemma. In the insects, however, he made the surprising observation that the intercellular network was in continuity with the tracheal system.

The second line of progress in the study of the reticular apparatus began with the discovery by Kopsch that it could be stained by prolonged immersion of the tissues in a two per cent. solution of osmic acid. In a short paper (Kopsch, '02) he described his method and contributed the results of his application of it. The results obtained corresponded very closely to those of Golgi but were obtained with greater certainty. Like Golgi he was unable to find any communications between the apparatus and the surface of the cell, although in addition to the osmic acid method he employed the resorcin-fuchsin method of Holmgren, to which reference will be made later.

The method of Kopsch has been exploited in particular by Misch ('03), and von Bergen ('04). The former found that the apparatus was not present in all cells and that in some cells it presented itself in the form of fragments, or of rows of granules. He found, moreover, in agreement with Golgi and Kopsch that the network never communicated with the surface of the cell, nor did it penetrate the nucleus. Von Bergen's studies extended to a very large category of cells ranging from wander cells to nerve cells. To show how general these structures are in animal cells a list of the elements in which von Bergen obtained positive results would have considerable interest. In addition to nerve cells he found a reticular apparatus in the following elements: prostate epithelium, pancreas cells, demilunes and mucous cells of the submaxillary gland of the cat, glandular epithelium from the trachea, chief cells of the fundus glands of the stomach, ciliated epithelium of the trachea, epithelium of the sweat glands, wander cells and many leucocytes, fixed connective tissue cells, cartilage cells, endothelium, smooth muscle, interstitial cells of the testis. The wide range of these observations taken in connection with the observations of Golgi and Cajal and their pupils indicate that the reticular apparatus is by no means a structure confined to a single cell category but is a cell organ of almost if not quite universal occurrence in the protoplasm of animal cells.

Before passing to a review of the investigations that have been made from the standpoint of the canalicular apparatus of Holmgren and others it may be of interest to refer briefly to the studies of Golgi on the development of the reticular apparatus. In the nerve cells of the fœtal calves of two or three months, he found the apparatus greatly reduced, often consisting of but a single fiber, with short branches running in various directions. In these cells the apparatus has a distinctly excentric position at one pole of the nucleus. In the new-born animal the net often extended around the nucleus, but left the perinuclear zone as well as the peripheral protoplasmic zone entirely free of such fibers. In old animals the apparatus was sometimes broken up into peculiar island-like fragments which however were connected with one another by single fibers. These observations suggest strongly that the apparatus constitutes a unit in its origin and developmental history.

The history of the intracellular canalicular apparatus, considered apart from the positive impregnations of Golgi and his followers, begins with the discovery by Holmgren ('99) of endocellular nets of juice-canals in nerve cells which he said was exhibited particularly well in preparations made from rabbit tissues. Almost at the same time Nelis ('99) described, in nerve cells fixed in sublimate or osmic acid and stained in iron-hæmatoxylin, etc., peculiar coil-like bands to which he gave the name "état spirémateux," the nature of which, however, remained to him fully obscure.

In a second publication (Holmgren, '99, 2) Holmgren described in greater detail the canalicular apparatus in the spinal ganglion cells of the rabbit, fixed in picric acid-sublimate and stained with toluidene blue and erythrosin. He found in these cells moderately fine canals of fairly uniform caliber which, anastomosing freely, formed a fairly dense network. The latter extended in general around the nucleus but often was found at one pole of the nucleus, more rarely at both poles. Here and there he found these canals communicating with pericellular canals, and at these points he was able to make out a distinct wall staining with erythrosin. He expressed the opinion that the canals were of lymphatic nature without however stating whether they were of extracellular or intracellular origin.

In 1899 Studnicka ('99) also described the canals in the protoplasm of the large ganglion cells of the trigeminus of *Petromyzon* and also in the spinal ganglion cells, in the nerve cells of the medulla oblongata and the cells of Reissner of the same animal. He explained the origin of the canals as due to the union of a row of vacuoles, and said that while many of the canals had smooth contours, yet in others might easily be seen the constituent vacuoles from which they had arisen. In a foot-note he remarked that he had not found in his objects the connection with extracellular structures described by Holmgren, although he admitted that the canals opened on the surface into the pericellular space.

In a series of papers dating from 1899 Holmgren has described the results of his investigations on this topic, covering a wide range of material including not only nerve cells, but cells from

various epithelia and from other sources. The existence of the canals has also been confirmed by a large number of observers including Kolster ('00), Fragnito ('00), Lugaro ('00), Donaggio (—), Pugnat ('01), Sjövall ('01), Smirnow ('01), von Bergen ('04) and others.

For comparison with the results of the Golgi and Kopsch techniques an enumeration of the different types of cells in which a canalicular apparatus has been found may be of interest. Holmgren demonstrated the canals in the following cells: gland cells of the pancreas and parotid, intestinal and gastric epithelium, epithelium of the epididymus, biliary duct epithelium, uterine epithelium, thyroid epithelium, liver cells, epithelium of the suprarenal gland. Retzius ('01) described similar canals in the giant cells of the bone-marrow, which, like Holmgren, he considered to be in direct connection with pericellular spaces.

It is to be noted that many of the objects studied by Holmgren coincide with those studied by Golgi and his pupils, and with those investigated by von Bergen, and that where this is the case, the canalicular structures described by Holmgren correspond closely in their location and in their configuration with those demonstrated by the other methods. Whatever conclusion we may reach with regard to the relation between the canalicular apparatus of Holmgren and the reticular apparatus in nerve cells, few who have studied the actual preparations made according to these different techniques in respect to epithelial cells and cartilage cells will deny their substantial identity. It is true that there are differences in the appearances obtained, but, in the opinion of many, these are sufficiently accounted for by the differences in the thickness of the sections studied in the different methods, and so, in the completeness of the apparatus which is brought to expression in a single preparation.

In his later papers dealing with these structures Holmgren has abandoned his original opinion that the canals are lymphatic in nature and constructed an entirely new hypothesis as to their nature. This hypothesis is based on the confirmation by him of the interesting observations of Nansen ('86) and Rhode ('91, '93, '95), that the nerve cells of certain Crustacea (Nansen, '86), and those of certain Gastropoda and Hirudinea (Rhode, *loc.*

cit.) were penetrated by a network derived from surrounding capsular cells. Holmgren found the nerve cells of *Helix pomatia* particularly suitable for the demonstration of these intracellular nets of capsular origin. He found here that the nerve cells were provided with a richer or poorer network of juice-canals, which were formed in the interior of a network of processes derived from other cells. He even found nucleated strands within the bodies of the nerve cell. In later publications (Holmgren, '01, '02, etc.)[1] he has developed this hypothesis on the basis of results obtained by the employment of a new method. He fixed his material in trichloracetic acid, or trichlorlactic acid, and stained it with a freshly prepared solution of Weigert's resorcin-fuchsin. By this method the protoplasm of the nerve cells stained faintly but that of the intracapsular cells stained dark violet, as did also the processes of the latter. By this means he was able to see processes of the darkly stained intracapsular cells which penetrated the nerve cells, branched within them, and anastomosed with one another, in order to produce an intracellular network. He applied this observation also to the nerve cells of vertebrates, and came to the conclusion that the latter were penetrated by processes of other cells which branched and anastomosed freely, to form in the interior of the nerve cells a "spongioplasma" which, however, in no wise belonged to the nerve cell, but was of extraneous origin. In the interior of these nets juice canals could arise, which communicated directly with similar spaces in the interior of the matrix-cells of this net. To this net of extraneous origin Holmgren gave the name "trophospongium." He regarded therefore the trophospongia not as fixed structures, but as undergoing a constant change, which depended upon the physico-chemical processes in the cell, and thought that, while at one moment the network of cell processes might sacrifice itself by liquefaction to the needs of the nerve cell, it might later be regenerated, by new growth of the process from without. He thus abandoned completely his former view that the canals represented circulatory or lymphatic structures, or a drainage system, in favor

[1]For complete references see Holmgren, E., "Neue Beiträge zur Morphologie der Zelle," in "Merkel-Bonnet Ergebnisse," Vol. 11, p. 275.

of the view that they represented the transitory phases of the reciprocal nutritive inter-relations of the capsular and nerve cells.

The object of the foregoing brief and incomplete résumé of the literature, has been to show that from three different lines of investigation we have evidence of the wide occurrence in animal cells, ranging in diversity from leucocytes to nerve cells and muscle cells, of a reticular apparatus, which exhibits itself in the form of a network of canals with colorless contents, or of a stained network according to the technique employed. The uniformity with which this apparatus has been discovered in those types of cells in which it has been sought justifies the expectation that similar methods will reveal similar structures in cells which have thus far not been investigated with this point in view. We are thus dealing with a cell organ of almost if not quite universal distribution in animal cells.

The question now arises—What is the significance of this structure?

The trophospongium theory of Holmgren, as far as I am aware, has found no support. Even if it were admitted for the nerve cells there are many categories of cells in which a reticular apparatus, or a canalicular apparatus is to be found, to which the theory is wholly inapplicable. For example, it is difficult to conceive how the reticular canalicular apparatus of the cartilage cells and of leucocytes, could be derived from the liquefaction of penetrating processes of other cells. Holmgren, it is true, has made an attempt to adapt his hypothesis to the canalicular apparatus of epithelial cells, and has described in the pancreas of the salamander the continuity of the intracellular network with intracellular strands which go to the periglandular connective tissue cells or to the centro-acinous cells. However, all of Holmgren's figures of preparations made by the trichloracetic acid, resorcin-fuchsin method are explainable on the basis of the canals having a precipitable content which when precipitated by the fixative, has an elective affinity for the dye. It is not by any means certain that the figures which Holmgren has given us of intracellular nets stained by fuchsin, in continuity with processes of capsular cells, do not really represent two different structures brought into apparent relation with one another by a common

affinity for the dye. It is certain, moreover, that the networks apparently composed of solid fibers demonstrated in the pancreas epithelium by the resorcin-fuchsin method, are not solid, for, in preparations made by this method, every cell in the section will show such a deeply stained network while sections of the same pancreas fixed in Kopsch's formol-bichromate solution and stained with iron-hæmatoxylin, will show in every cell a system of canals with unstained contents.

Accordingly, we must either reject Holmgren's hypothesis or assume that there are two sorts of these nets, those of cartilage cells and epithelial cells and leucocytes being different from those of nerve cells.

The statement of Legendre ('08–'09) that these structures are either wholly absent or are the result of pathological changes is not to be seriously considered, in view of the fact that this author in attempting to explain the positive observations in this regard of so many experienced investigators, is compelled to resort to the wholly unwarranted assumption that their results have been due to the selection of unhealthy animals, or to the fixation of tissues after several days of inanition, or several hours after death.

Many observers, including Retzius ('09), are inclined to believe that they represent an intracellular system of nutritive or drainage canals having direct relations with the lymphatic system. The extracellular communications are, however, denied by Golgi and his pupils, who have never seen them in their preparations made by the chrome-silver impregnation methods, nor by the silver reduction method. They are equally denied by von Bergen, who, however, admits the existence of canals in no wise connected with these, which he regards as artefacts, which do open on the surface of the cell.

Von Bergen ('04) who studied these structures by all three methods but, in particular, by the osmic acid method of Kopsch, agrees with Golgi that the structures are for the most part networks of fibers composed of a substance which reduces osmic acid, but explains the discontinuous elements found by him in many cells studied, by the assumption that they represent different stages in the formation or destruction of the apparatus

which thus would have a variable structure from moment to moment in the cell. He claims that the reticular apparatus arises by the appearance in the cell protoplasm of granules or droplets which arrange themselves in net-like or tortuous rows which fuse to form more continuous fibers, and further, that the network so formed can undergo vital changes, by virtue of which it loses its stainability and becomes dissolved, the canals so formed finally disappearing by absorption of their contents.

In view of the almost universal occurrence of these structures in all of the tissue cells of mammals, and in many of those of lower vertebrates and invertebrates, it seemed probable that they would not be wholly absent from the cells of the other great division of living organisms, namely from the cells of plants. Accordingly, I have studied with this end in view the structure of certain plant cells, using for this purpose in addition to the conventional methods of plant histology, those methods which in my experience were best for demonstration of the canalicular system in animal cells. It seemed probable, in view of the conditions found in the animal cell, that, if a homologue of the canal network of the animal cell were to be found in plant cells, it would be studied with greatest ease in those plant cells in which the vacuolar system had not yet reached its full development, namely in meristem tissues, sporogenous tissues and their products, cambium and embryonic tissues. The three last, however, did not lend themselves readily to this investigation because of the difficulty introduced by the slow penetration of the fixing agents, so that I have been obliged for the present to content myself with the results obtained in the root-tips of *Allium*, *Lilium* and *Iris*, and in the tapetum of the lily. Whether the consistent results obtained from the study of these cells are generally applicable or not to plant cells, future investigation will show. In the meantime, because of the fact that the results are at variance with the accepted views of the structure of the cells in question, because they furnish a new interpretation of the history of the vacuole of these cells and in particular because they seem to throw an interesting light on the question of the nature of the canalicular apparatus of animal cells, it seems wise to put these preliminary observations on record.

The descriptions which follow have been drawn largely from the study of preparations of the root-tip of the onion, but the observations made on the roots of the other genera mentioned are in full accord with them.

In his study of the vacuolar system of the cells of plants, Went ('88) describes the young cells of the onion root-tip as follows: "In the youngest cells of roots two or three millimeters in diameter I saw a great number of very small vacuoles; the largest had a diameter of four mikra, the smallest of one mikron." For these small vacuoles he claims reproduction by division, in the sense of the tonoplast theory of DeVries ('85). He also derives the vacuole of the older cell from these multiple vacuoles, by a process of coalescence.

In preparations made after fixation in Flemming's strong fluid, Hermann's fluid, Zenker's fluid, Carnoy's fluid, etc., and stained in iron hæmatoxylin, or in the three-color process of Flemming, results exactly corresponding to these were obtained, that is to say, the young cells contained a multitude of small vacuoles which by their coalescence seemed to form the large central vacuole of the older cell.

On the other hand, preparations made by methods which I had found to be most effective for the demonstration of the canalicular apparatus in animal cells, gave results which were wholly different. In these there was no trace in the youngest cells of the root tip of the multiple vacuoles described by Went and others, but instead, each cell possessed an intricate network of canals the component elements of which in the youngest cells were often of extreme fineness. These canals were best seen in the dermatogen cells on the surface of the root but were recognizable as such, though less well preserved, in the cells of the plerome. Fig. 1 shows a cell in which this system is composed of extremely fine canals. In this figure it will be seen that the canalicular system tends in these cells as in the animal cells to

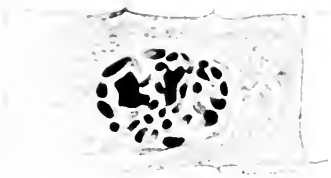


FIG. 1. Cell of outer layer of the root tip of onion, showing fine canals in the cytoplasm. × 800.

leave a peripheral zone of cytoplasm wholly free from the canals which constitute it. In Fig. 2 are shown four of the large wedge-shaped cells from the region of most active division of the dermatogen, in which the type of the canalicular system is well brought out. Here it will be seen that in these plant cells, as in the animal cells, the network tends to be concentrated on one side of the nucleus, and, as in the epithelial cells, this point of concentration is not one of the division poles of the nucleus but

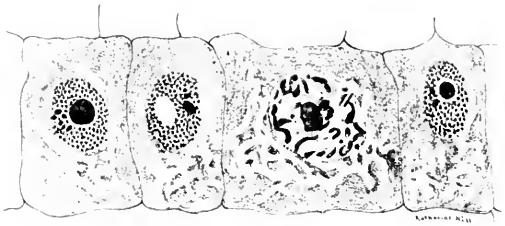


FIG. 2. Four cells of outer layer of root tip of onion, showing more advanced condition of the cytoplasmic canals. × 800.

corresponds to one side of the equator of the future spindle. The system constitutes a closed system of canals, lying in very close relation to the nucleus, never, however, invading, in dividing cells, the spindle territory. From this network run out branches which end freely often near the cell wall in a small expansion. Many of the canals in some preparations, and this is particularly true of the smallest canals, such as those shown in Fig. 1, show moniliform enlargements, as if they were on the point of breaking up into a row of vacuoles, or possibly, as if they had just been formed by the coalescence of a row of vacuoles. Again frequently, the canals show a spiral or tortuous course, as if they were fixed while in a condition of internal tension, which resembles very closely the spiral or tortuous condition found in many nerve cells (état spirémateux of Nelis).

Tracing this system in the older and older cells of the root tip it is found that as the cell retreats from the growing point, the canals become progressively larger and larger. In the intermediate stages of this process the condition depicted in Fig. 3 is obtained. Here there is still a continuous system of canals but they are fewer in number and broader than in the younger

cells. Ultimately by a continuation of this process we have the familiar picture of the plant cell with a large central vacuole across which run strands of protoplasm which are the last attenuated remains of the protoplasmic partitions between the canals.

A similar mechanism is revealed by the same technique in the tapetal cells of *Lilium candidum*. In preparations made after fixation in Flemming's fluid, the protoplasmic tip of the cell presents a foam-structure owing to the presence in it of a

FIG. 3. Five cells of second row of free tip of anther, showing the expanded canals, beginning to form the large central vacuole.

large number of fine vacuoles. In preparations, however, which have been made by the technique referred to above, it is seen that instead of a multitude of minute vacuoles or alveolæ, there is a system of exceedingly fine canals forming a network which opens at intervals into the large vacuole which occupies the base of the cell.

Apart from the elements which constitute the tubes or vacuoles according to the method of preparation, the cytoplasm of these cells shows no indication whatever of an alveolar structure under the microscope. It is composed of an optically homogeneous ground substance in which are imbedded the mitochondria and other granular elements, for example plastids, which may be present.

The two different techniques, therefore, give us two entirely different conceptions of the history of the vacuole. According to the first the vacuole arises from the coalescence of preëxisting innumerable small vacuoles. According to the new methods, the vacuole in these cells constitutes a unit element from the very beginning, being represented in the younger cells by a single system of anastomosing canals.

The decision as to which of these views expresses the condition in the living cell must of necessity rest on the examination of living cells. Before taking up this question, however, we may discuss the significance of these observations in the interpretation of the canalicular apparatus of the animal cell for this interpretation does not of necessity imply the assumption that the canalicular structure so demonstrated in the plant cell has a real preëxistence in that form in the living cell. We may, on the contrary, treat the technique as an experimental method and discuss the results comparatively on this basis.

For the demonstration of the vacuolar system of plant cells as a network of canals I have found the following fixing fluids best adapted:

1. Formaline, Bichromate, Sublimate.

Neutral formaline (freshly distilled)	10 c.c.
Water	90 c.c.
Potassium bichromate	2.5 gr.
Mercuric chloride	5 gr.

2. Kopsch's Fluid.

Potassium bichromate 2.5 per cent. in water	75 c.c.
Neutral formaline	25 c.c.

With these fluids, as indicated above, the cells of the root tip show a network of canals, whereas the same tissues fixed in Flemming's solution show, instead of canals, multiple small vacuoles. The same statement holds good for animal cells similarly treated. For example, the epithelial cells of the intestinal glands fixed in the formaline-bichromate-sublimate mixture, or in Kopsch's fluid, show a beautiful canalicular system, while the same cells fixed in Flemming's fluid show at the site of the canals merely a large number of exceedingly fine vacuoles. Thus whether we accept the multiply vacuolated condition, or the canalicular condition, as the preëxisting one in the living cell, the analogy between these structures in the animal and vegetable cell holds.

On the basis of the similarity in constitution of the canalicular apparatus of the plant cell to that of the animal cell, and of the similarity in behavior of this system when treated by the same methods and an account of the part these canals in the plant cell

take in the history of the vacuole of the latter. I think we are justified in stating for the present, to be sure, only as a working hypothesis, that the network of canals found in so many animal cells is the physiologic and morphologic equivalent of the vacuolar system of the plant cell.

We may now return to the consideration of the question whether the canalicular system represents the true structure of the vacuolar mechanism of young plant cells or not. This question can, of course, be answered only by observations of the living cells themselves, and the investigation of these is beset by extraordinary difficulties in the case of the plant cell due in particular to the impossibility of finding a solution in which to examine the cells, which is not itself injurious. In the effort to find a suitable fluid for this purpose I tried solutions of potassium nitrate, of sodium chloride, and of cane sugar of different concentrations, but found in all that the surface layers of cells showed rapid changes of the structure of the protoplasm which made it difficult to study the presumably uninjured deeper layers of the sections. I was finally obliged to resort to the expedient of using as a mounting medium the freshly expressed juice of similar tissues, although even in this the cells of the surface layers of free-hand sections underwent more slowly the same change. In these surface layers so mounted the cells showed the multiple vacuolar condition described by Went. In the deeper layers, on the contrary, in sections of the onion root tip, one could see, with difficulty to be sure, but still unmistakably the canal system represented in Figs. 1, 2 and 3. As these cells are watched, however, the canals are seen to break up slowly into rounded vacuoles thus bringing about the condition generally recognized in these cells. I regard, therefore, the canalicular system as the true condition intra vitam of the vacuolar apparatus in these cells of the root tip, and believe that the multiply vacuolated condition is of secondary origin due in most cases to injury of the cell.

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A STUDY OF CHROMOSOMES OF TOXOPNEUSTES VARIEGATUS WHICH SHOW INDIVIDUAL PECULIARITIES OF FORM.

BARBARA HEFFNER.

Introduction.

The observations described in this paper were made during the winter and spring, 1909–10, in the Biological Laboratory of Bryn Mawr College.[1] Last November after my arrival in Bryn Mawr Professor Tennent suggested to me a study of the chromosomes in eggs of certain echinoderms with special reference to characteristic peculiarities of form, a question especially significant since the appearance of Baltzer's ('09*a*) paper on the chromosomes of *Strongylocentrotus lividus* and *Echinus microtuberculatus* which has thrown new light on the individuality of chromosomes in echinoids. Previous to this author's first publication on the subject ('08) there existed only a suggestion by Boveri ('01 and '07) that in some echinoids there occur chromosomes of a characteristic shape. Baltzer pointed out that in *Echinus microtuberculatus* there are two very long rod-shaped chromosomes, two long hook-shaped ones, and two or three horseshoe-shaped chromosomes, while in *Strongylocentrotus lividus* there are two long rod-shaped chromosomes, two long hook-shaped ones and in a part of the eggs one smaller hook-shaped chromosome. As this latter one occurs only in a part of the eggs—in about one half of them—Baltzer suggests ('09*a*, p. 596) that it is probably an idiochromosome whose smaller mate is one of the shorter rod-shaped chromosomes. The same suggestion is made in regard to one of the horseshoe-shaped chromosomes in *Echinus*, in cases where there are three of that type. While idiochromosomes and other heterochromosomes have for some time been known in insects, arachnids and myriapods,

[1] I take advantage of this opportunity to express my sincere thanks for the generous scholarship awarded to me by Bryn Mawr College and for encouraging suggestions from Professor Tennent and Dr. Stevens during the course of my work in their laboratory.

they have only quite recently been discovered by Gulick in the nematode *Heterakis* (Boveri, '10), by Baltzer in the echinoids, and by Guyer ('09*a* and *b*) in vertebrates.

Observations on metakinesis stages (Baltzer '09*a*, Plate XXXVII., Fig. 9) have shown that the hook-shape of certain chromosomes in *Strongylocentrotus* is due to the fact that the spindle fiber from each pole is attached at a point about one third of the length of the chromosome from one end, so that a shorter and a longer arm are formed. In the horseshoe-shaped chromosomes in *Echinus* the fiber is attached about half way between the two ends, so that the two arms are nearly of the same length (*ibid.*, Fig. 10). As for the origin of the hook-shaped and horseshoe-shaped heterochromosomes, observations on cross-fertilized eggs, $\frac{\textit{Strongylocentrotus}\ \male}{\textit{Echinus}\ \female}$, and on multipolar mitoses of *Strongylocentrotus* have shown that they come from the female pronucleus, the corresponding pair in the male being rod-shaped. Apparently the female has an unequal pair of heterochromosomes, one hook- or horseshoe-shaped, the other rod-shaped; while the male has a corresponding equal pair of rod-shaped chromosomes. It may be mentioned that this is the reverse of what is found in insects, but as in most insects the female nucleus must obtain more chromatin than the male nucleus.

Material and Methods.

My observations were made upon eggs of *Toxopneustes* and *Arbacia*, collected and preserved by Professor Tennent. As preserving fluid either picro-acetic or sublimate-acetic was used. Sections of 5 μ thickness were stained with Heidenhain's iron haematoxylin, except in a few cases mentioned later.

The figures are all drawn with Abbe's drawing camera, Zeiss oil immersion 2 mm. apochr. objective, oc. 12, enlarged to twice or four times the original diameter and reduced one half.

Arbacia punctulata.

The eggs of this species are quite unfavorable for detailed cytological studies. Not only are the chromosomes very small but the cytoplasm of the egg is filled with pigment granules so

that a sharp differentiation of plasma and chromosomes is impossible. Following Dr. Stevens's suggestion, I tried to bleach the section with H_2O_2, a method successfully applied in some other cases, but entirely useless in *Arbacia*. I therefore gave up further study of the chromosomes of this species.

Toxopneustes variegatus.

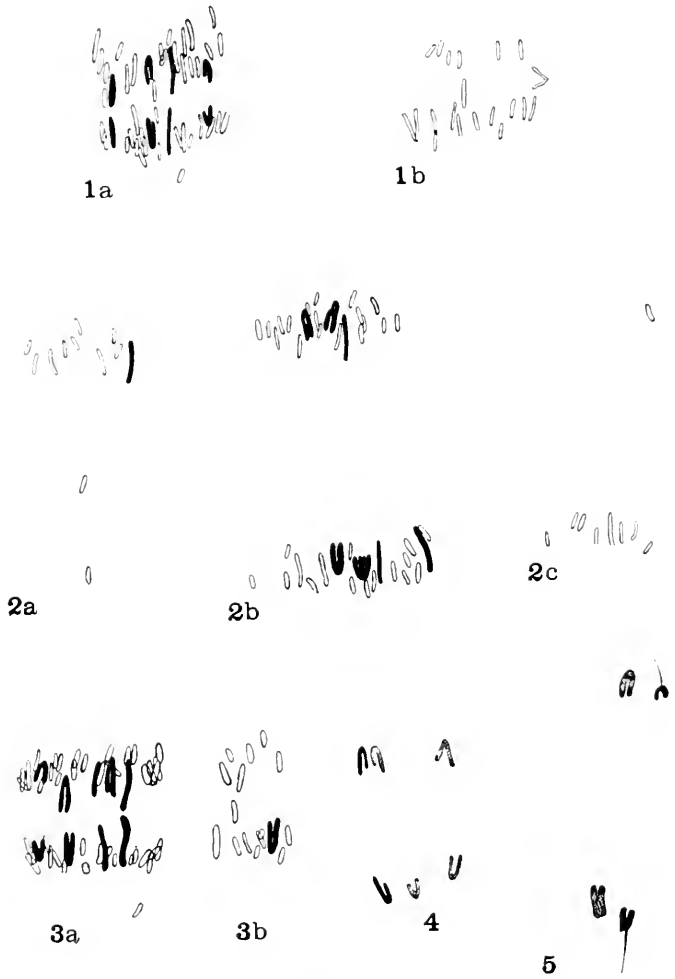
The observations were made on two series of eggs from two different localities, one from Beaufort, N. C., the other from the Tortugas. The results obtained are the same for both. I began with the study of the chromosomes in the first segmentation spindle and found in *Toxopneustes* as Baltzer ('09*a*) had done in *Strongylocentrotus* and *Echinus* a considerable variation in the length and form of the chromosomes.

As in *Echinus* and *Strongylocentrotus* there are two extremely long chromosomes in each daughter plate. Their behavior resembles that described by Baltzer in that these long rod-shaped chromosomes are often late in splitting and moving to the poles (compare Text-fig. 3, *a*, with Baltzer's '09*a*, Pl. XXXVII., Fig. 5, *a* and *b*). These chromosomes may also be seen in my Figs. 1, 2 and 3. Sometimes these long chromosomes are contracted more and then appear thicker and shorter (Fig. 1, *a*, the left pair of long rod-shaped chromosomes).

A type of chromosome of peculiar shape, found in all of the eggs, is one which is usually V-shaped, but sometimes more horseshoe-shaped (Figs. 4 and 5). Very frequently in late anaphases the two arms are parallel or nearly so, and one may partly overlie the other, but there is hardly ever any doubt as to whether there is one chromosome with two arms present or two separate rod-shaped ones (Fig. 3, *a* and *b*). As may be observed from Fig. 4 the length of the two arms in these chromosomes may vary slightly when the arms are lying in one optical plane. I tried to determine whether this difference is confined only to certain chromosomes or to chromosomes in certain eggs, but no regularity seems to exist. The probable origin of the difference in length will be discussed later.

These V-shaped chromosomes occur in all fertilized *Toxopneustes* eggs and there are either two or three present. Among 34

one-cell stages examined with reference to this point, I found in 16 cases two, and in 18 three such chromosomes. Fig. 1, *a*, illustrates a case with two V-shaped chromosomes in each daughter plate. Their antagonistic position proves that they are division



FIGS. 1–5.

products of the same chromosome, their regular number, that they are not merely incidental features. The other chromosomes are very crowded, as most of them occur in one section. Their position has been slightly changed in cases where they were

over or under the V-shaped chromosomes, and this holds for all similar figures. Special care was of course taken to keep the position of the long rod-shaped and the V-shaped chromosomes in every case as accurately as possible.

Fig. 2, *b*, shows daughter plates of a late anaphase where there are three V-shaped chromosomes with more nearly parallel arms. Two of these chromosomes are very close together, one partly overlying another in each plate. Fig. 3 also shows in *a* and *b* three V-shaped chromosomes in early anaphase. One pair of these chromosomes appears smaller than the two others, but in examining other cases I found no regularity in the apparent size of the three pairs. Sometimes all three pairs vary a little, sometimes they are all of apparently the same size, sometimes only the members of one pair vary in apparent size. This difference may be due to difference in contraction or to an original difference in length of the chromosomes. As Baltzer's ('09*a*, p. 568) measurements of the hook-shaped chromosomes show, the length of chomosomes of a certain type is quite variable; it may vary from 9.75–14.0 mm. There is no resemblance however to the conditions in *Strongylocentrotus* in respect to the third pair of hook-shaped chromosomes which always is considerably smaller than the other two.

One of the three V-shaped chromosomes is probably a heterochromosome, as Baltzer assumes to account for the conditions found in *Strongylocentrotus* and *Echinus*. Studies in ovogenesis and spermatogenesis would be necessary to obtain evidence for or against the suggestion made.

As it is difficult to count the total number of chromosomes in a lateral view of the daughter plates I counted the number in polar views where fortunately the V-shaped chromosomes show very clearly.

Fig. 6, *a* and *b*, represents two succeeding sections through two anaphase daughter plates; the V-shaped chromosomes are the two double ones, finished in solid black. The number of chromosomes in each plate is 36. Fig. 7 shows a polar view with three V-shaped chromosomes; here also the number is 36.

I counted 14 anaphase plates from the pole, with a clear arrangement of chromosomes, and found the average number

36, always counting the two arms of the V-shaped chromosome as one.

Although I did not have very much material I was nevertheless able to trace the V-shaped chromosomes in the 2-, 4-, 8-, 12- and 16- to 32-cell stages.

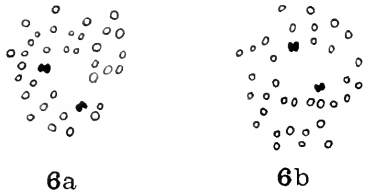


FIG. 6.

FIG. 7.

Fig. 8, *a* and *b*, shows a metaphase from a 2-cell stage, with the three V-shaped chromosomes distinguishable by their characteristic splitting figures, which are fully explained below.

Fig. 9, *A* and *B*, represents two adjoining cells of a 16- to 32-cell stage. In cell *A*, 9*b*, we see three V-shaped chromosomes

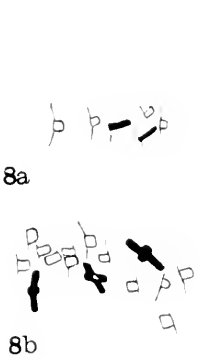


FIG. 8.

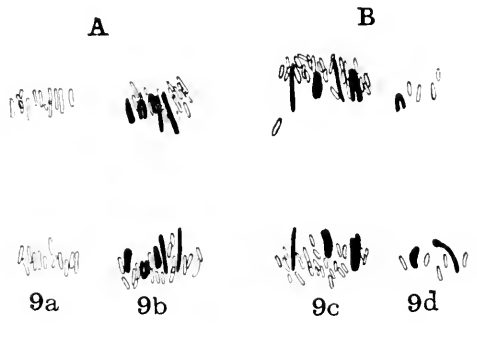
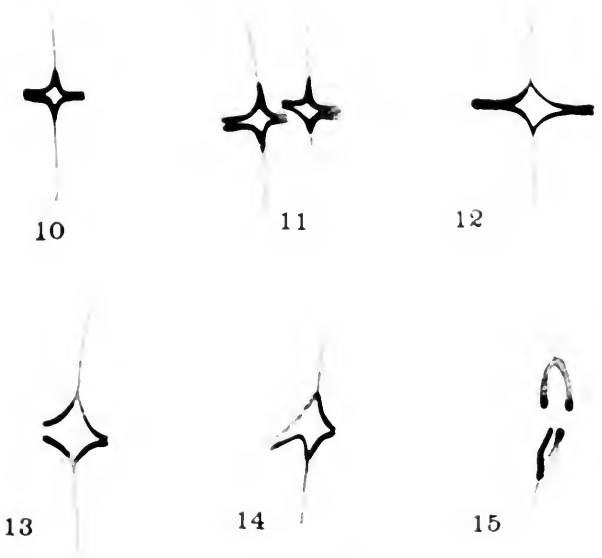


FIG. 9.

and the two long ones. In *B*, 9*c*, we see again two of the V-shaped chromosomes; and in 9*d* the third one. The two long ones are distributed between *c* and *d*. The chromosomes in this stage are so small and crowded together that an accurate count of their total number is impossible.

The figures 10–15, enlarged 4 diameters and in publication reduced one half, show splitting, or metakinesis, stages of the

V-shaped chromosomes. From these figures it is evident that the V-shaped chromosomes of the anaphase come from a long rod-shaped chromosome to which the spindle fibers are attached with more or less regularity in the middle of the chromosome (Fig. 12). If the separation of the daughter-chromosomes takes place earlier at one end than at the other (Fig. 13) or one arm of the chromosome is contracted more than the other, or, as Fig. 14 suggests, if some hindrance on one side prevents one



FIGS. 10–15

end from moving toward the pole as rapidly as the other end, then the variations in the length of the arms mentioned above (Fig. 4) appear. In comparing these figures with Baltzer's '09*a*, Pl. XXXVII., Fig. 10, we cannot fail to find a close resemblance.

In Baltzer's paper '09 *a*, p. 607, we notice a suggestion that in the hook-shaped chromosomes there may be a union of two chromosomes, end to end at the point where spindle fibers are attached. The reasons given for this suggestion are the extraordinary length of the hook-shaped chromosomes, and the fact that all other chromosomes are attached to the spindle fibers by one end. This suggestion is a very natural one, for such apparently homogeneous but plurivalent chromosomes are

known in *Ascaris*, and compound chromosomes are found in the maturation mitoses of certain insects (McClung, '05, Payne, '09). In some of these latter cases (Payne, '09) the plurivalent chromosome has a spindle fiber attached to each unit in the early metaphase and in many cases two spindle fibers from each pole are attached to one of each of the four units of a tetrad in primary maturation mitoses (Stevens, '10).

Since the V-shaped chromosome of *Toxopneustes* seems to be exactly comparable to the hook-shaped chromosome of *Echinus* and *Strongylocentrotus*, the question arose whether, assuming that the V-shaped chromosomes of *Toxopneustes* may be bivalent, one might by careful observation be able to trace in the early metaphase two spindle fibers from each pole attached to each of them. As the spindle fibers were not especially clear in the preparation stained with iron-hæmatoxylin alone, a few slides were counter-stained with Rubin S. Among 42 cases I found only two where I was inclined to count two fibers; in all other cases I was certain that only one fiber from each pole was attached to each V-shaped chromosome. My observations have therefore failed to add any facts supporting Baltzer's suggestion, which, however, future investigation may verify.

Baltzer ('09*a*) traced the heterochromosome in *Strongylocentrotus* and *Echinus* to the female pronucleus. Unfortunately I was not able to obtain suitable material for this purpose, but further investigation will probably reveal the same conditions as in *Echinus* and *Strongylocentrotus*.

Discussion.

Comparing the chromosomes with peculiar shape in *Echinus* and *Strongylocentrotus* with those in *Toxopneustes* we find that the hook-shaped chromosomes in *Echinus* and *Strongylocentrotus* have no exact equivalent in *Toxopneustes*. The two extremely long rod-shaped ones are found in the three species. The V-shaped chromosomes in *Toxopneustes* are very similar to the horseshoe-shaped chromosomes of *Echinus* in respect to their formation and the equal length of their two arms. They differ from the *Echinus* chromosomes as already mentioned in their length and slenderness. As in *Echinus* we are not able to

distinguish a particular one of these three V-shaped chromosomes as a heterochromosome.

The discovery of individuality of form among the chromosomes in echinoids is a very valuable factor in support of Boveri's "Individualitätstheorie" of the chromosomes. One also welcomes every such means of distinguishing parental chromosomes in cross-fertilized eggs. *Toxopneustes* for instance has been used for cross-fertilization (Tennent, '07 and '10). These chromosomes which show marked individuality of form will be of special value in cases of cross-fertilized eggs, where, as shown by Herbst ('09) and Baltzer ('09*b*), the chromosomes of one parent are almost entirely eliminated during the first segmentation divisions.

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ON THE INHERITANCE OF COLOR IN THE AMERICAN HARNESS HORSE.

A. H. STURTEVANT, JR.

In a study of the English thoroughbred horse C. C. Hurst[1] has shown that chestnut is recessive to bay and brown. He supposes that the presence of black in the coat is the dominant character. Now black, gray and most roan horses also have black in their coats, but 95 per cent. of the English thoroughbreds are bay, brown or chestnut, so that Hurst was unable to verify his supposition. The American trotting and pacing horse, however, a close relative of the English thoroughbred, exhibits colors in proportions much more favorable for an investigation of this kind. These proportions are about as follows: bay, 53 per cent.; black, 13 per cent.; brown, 15 per cent.; chestnut, 14 per cent.; gray, 3 per cent.; roan, 2 per cent.; dun, .1 per cent.

Perhaps before going further it will be well to give a brief discussion of these colors. According to Miss F. M. Durham, as quoted by W. Bateson,[2] there are three pigments, yellow, black and chocolate, concerned in the color of horses, as in mice, rabbits and other animals. Chestnuts have the yellow pigment alone.[3] Bays have both yellow and black pigments, and browns are only very dark bays, shading into the self-colored blacks on the other extreme. Grays have black hairs mixed with white ones, usually in a dapple pattern. Roans are of at least three types. The most common are the bay, red or strawberry roans, which have yellow-black hairs intimately mixed with white ones. The black, blue or gray roans appear to differ from grays chiefly in that their black and white hairs are more intimately mixed. The chestnut roans have yellow and white hairs. As will appear later the fact that there is no black in this class introduces a possible source of error into my calculations. However, these chestnut

[1] *Proc. Royal Soc.*, Vol. 77, B., 1906, p. 388.

[2] "Mendel's Principles of Heredity," p. 125.

[3] According to Bateson some chestnuts are really chocolates, but these are like the yellows in having no black.

roans are rare, forming something less than 10 per cent of the total number of roans seen on the streets of New York, and nearly all of those seen are heavy draught horses, so that I feel sure they are very rare indeed among blooded trotters. The official records do not distinguish between these three types of roans, but in the journals it is not rare to see a horse described as belonging to one of the two commoner classes, though I have never yet seen in them a reference to a chestnut roan. There are several types of duns, but, as appears in the list above, all are rare. In a few families duns seem to be dominant to bay, brown and black, and one was connected with gray, but beyond this I have found nothing about the color. Lastly there are a very few spotted trotters, but these are all poorly bred ones, with short pedigrees, and I have done nothing with them.

I have tried to show here that Hurst's discovery of the dominance of bay and brown to chestnut holds good for the American harness horse, and that gray, black and roan, or all other colors containing black, are also dominant to chestnut. In order to avoid confusion I shall call this dominant factor for the presence of black Hurst's factor. Apparently all trotters have the factor for chestnut, which I shall represent by *C*. This factor is hypostatic to all the others here mentioned. The factor next highest in the scale is that for black, or Hurst's factor, *H*, its absence being *h*. Next higher is that for bay, *B*, its absence being *b*. At the top stand the gray and roan factors, *G* and *R*. Now most horses have neither of these last two, and are therefore *ggrr*. A chestnut will always be *CHh*, but may have any combination of the other factors and their absences, since they produce no visible effect in the absence of Hurst's factor. Self blacks are *CHHbb* or *CHhbb*, since bay is epistatic to self black. Bays have one or two *C*'s, *H*'s and *B*'s. Grays have *C*, *H* and *G*, and roans have *C*, *H* and *R*. Whether these last two must have *B* or not is not clear. I shall discuss the three epistatic colors more fully and give my theory as to brown when I have presented the evidence as to Hurst's factor.

The chief authorities for the statistics and color pedigrees given here have been Wallace's "Year Book of Trotting and Pacing" and Wallace's "American Trotting Register," both of which are official records.

It is a recognized fact among the breeders of harness horses that certain stallions never produce chestnut foals. In *Wallace's Monthly* for February, 1880, there is an article by "Truth," in which he says: "I have learned that neither of the brothers [Volunteer and Sentinel] have ever sired a chestnut colt." W. H. Marrett, in the September, 1890, issue of the same paper, tells us that the two bay sires Volunteer and Electioneer never had chestnut foals. Both were by a bay sire (Rysdyk's Hambletonian, which appears in the first table below), one being from a bay mare, the other from a brown. In a sale catalogue issued in 1903 C. W. Williams says of the brown stallion Belsire: "His get are . . . bays, browns and blacks." This horse is a son of the Electioneer mentioned above and of a black mare whose sire was a black and dam bay. He is a full brother to the bay Chimes which appears in the table below, and to Bow Bells, bay, and St. Bel, black, both also probably homozygous.

I have found a good many sires homozygous for Hurst's factor. The small number of gray and roan sires in the table below is to be explained by the small number of those colors existing. It will be noticed that two of the number have one chestnut foal each recorded. Director's was found in an advertisement in a horse journal—obviously a poor authority, as the pedigree might easily have been false. That by Jay Bird is Cardenas, trotting record 2:26¼, from a chestnut mare. He is recorded as a chestnut by the "Year Book." But the "Year Book" does sometimes make mistakes in the matter of color. Among others I could mention is the case of the bay stallion Charley Wilkhurst, recorded as a gray gelding.[1] In this connection it is worth noting that Hurst found about 1 per cent. of exceptions recorded in his investigation, but was able to explain most of them by showing them to be probably mistakes.

[1]See *The Horse Review* for December 12, 1905, p. 1424.

TABLE OF SIRES HOMOZYGOUS FOR HURST'S FACTOR.

Stallion.	Color.	Sire's Color.	Dam's Color.	Foals.						
				Bays.	Blacks.	Browns.	Chestnuts.	Grays.	Roans.	All.
Chimes[1]	bay	bay	black	15	1	4	0	0	0	20
Electioneer[2]	bay	bay	brown	39	2	9	0	2	0	52
Happy Medium	bay	bay	bay	59	0	4	0	4	2	69
Rysdyk's Hambletonian	bay	bay	brown	40	0	8	0	2	0	50
Director	black	brown	bay	15	35	19	1	2	2	74
Gambetta Wilkes[3]	black	brown	brown	12	12	6	0	0	0	30
Oro Wilkes	black	black	black	15	3	4	0	0	0	22
Sable Wilkes	black	bay	black	23	19	7	0	0	0	49
Dictator	brown	bay	black	12	7	5	0	0	0	24
George Wilkes	brown	bay	brown or roan	12	5	3	0	0	1	21
Prodigal	brown	bay	bay	94	0	19	0	3	0	116
Conductor	gray	bay	gray	3	1	5	0	15	0	24
Pilot Medium	gray	bay	gray	39	0	5	0	61	1	116
Eagle Bird	roan	roan	brown	15	1	3	0	0	8	28
Jay Bird	roan	brown	roan	54	0	15	1	3	63	136
Margrave	roan	brown	roan	28	0	5	0	2	30	65

[1]Half of these foals from mares by the chestnut Mambrino King.

[2]Twenty-nine of these chestnut mates.

[3]Eleven of these from chestnut mates.

I have found only 69 cases of chestnuts being mated together, but in all these the result was chestnut. They are from the following stallions: .

Chestnut Sire.	Color.		Number of Chestnut Foals from Chestnut Mates.
	Sire.	Dam.	
Nutwood	bay	gray	10
Robert McGregor	bay	bay	7
Attorney	bay	?	6
Daniel Lambert	bay	?	5
Elyria	chestnut	black	4
Hinder Wilkes	bay	chestnut	4
Mambrino King	black	?	4
Six different sires with two each			12
Eleven sires with one each			11
Total			69

The finding of foals from heterozygous sires and chestnut mares is a very slow and laborious task, but I have found some from the following stallions:

Stallion.	Color.	Foals from Chestnut Mares.	
		Chestnuts.	Total Found of all Colors.
Alcyone	bay	3	7
Axtell	bay	8	10
Boreal	bay	6	8
Guy Wilkes	bay	7	8
Norval	bay	1	6
Onward	bay	8	20
Red Wilkes	bay	8	14
Strathmore	bay	3	6
Total bay sires	bay	44	79
Grattan	black	1	4
Simmons	black	5	7
Total black sires	black	6	11
Alcantara	brown	2	8
Allerton	brown	8	17
Total brown sires	brown	10	25
Alcryon	gray	2	2
Re-election	gray	3	4
Total gray sires	gray	5	6
Grand Total		65	121
Expectation		60½	121

Here again I have been handicapped in working with gray and roan by the small number of sires of those colors, and also by the fact that most of the best known of those existing seem to be homozygous. However, I have found some sires of those colors which throw a fair percentage of chestnut foals, as shown in the following table, which shows all known foals. Two blacks are also included.

Stallion.	Color.	Chestnut Foals.	Foals of All Colors.
Bellini	black	3	17
Mambrino Patchen	black	3	16
Alcryon	gray	9	30
Pilot, Jr.	gray	4	20
Jay Hawker[1]	roan	1	20
Roan Wilkes	roan	3	10
Tom Hal, Jr.[2]	roan	1	15

[1] The one chestnut from Jay Hawker is scarcely to be doubted, as he is Country Jay 2:07¼, world's champion trotter under saddle, and one of the most prominent race horses of the season of 1909.

[2] This one chestnut also is not a doubtful one. His name is in fact Chestnut Hal.

It seems to me that we have here sufficient proof that Hurst's original supposition is right—that the dominant character is the black in the coat. However his idea that the black, if present, exists on the fetlocks, seems to me to be unjustified, as Joe Patchen, a heterozygous black, has white feet, though the white does not reach as far up as the fetlock on the left hind one. Grattan, another heterozygous black, also has three white fetlocks. It is not at all rare to see pictures of bays or browns with one foot or more white as far up as the fetlock or further. Here are some examples: bays Capo, both hinds; Moko, right hind; Ario Leyburn, left hind; Allerworthy, both lefts; Hail Cloud, right front; browns Redlac, both hinds; The Harvester, left hind; Searchlight, both hinds. At least one of these (Moko) is homozygous.

In the case of the next factor, bay, a complication arises in regard to brown. As explained before the presence of bay is dominant to its absence, and the color next below it in the scale is black. Brown is a color between these two, shading into both extremes. It seems that brown is usually a heterozygous color, represented by *CHBb*, but that bay also is quite often heterozygous, and that brown may occasionally be *either* of the homozygous types, *CHBB* or *CHbb*. This suggests the idea that the line between black and bay should be drawn somewhere near the black limit of brown. The obvious result of this complication is the creation of considerable confusion in the numerical proportions of the two colors. It is evident that, except for this complication and the appearance of some chestnuts, bay will act as though it were an ordinary dominant to black.

Below is a table showing twelve sires homozygous for the bay factor. Six of them are bays, and one is the only brown certainly known to be homozygous for this factor. Two are chestnuts, and therefore lack Hurst's factor. The other three are a gray and two roans, and it will be noted that all three of them appeared in the table of sires homozygous for Hurst's factor. They are not bays because they also bear other factors, as I shall explain later. Of the bays two are homozygous and four are heterozygous for Hurst's factor. The single black from Robert McGregor is Bobby Good, pacer, 2:11¼, out of a daughter of Ashland Wilkes.

Stallion.	Color.	Sire's Color.	Dam's Color.	Foals.						
				Bays.	Blacks.	Browns.	Chestnuts.	Grays.	Roans.	All.
Ashland Wilkes	bay	bay	?	90	0	0	19	5	0	114
Rysdyk's Hambletonian	bay	bay	brown	40	0	8	0	2	0	50
Happy Medium.	bay	bay	brown	59	0	4	0	4	2	69
Onward.....	bay	brown	bay	85	0	7	24	1	0	117
Red Wilkes.....	bay	brown	?	92	0	7	22	3	0	124
Sphinx.....	bay	bay	chestnut	30	0	5	9	4	0	48
Prodigal.....	brown	bay	bay	94	0	19	0	3	0	116
Axworthy.....	chestnut	bay	bay	33	0	2	32	1	4	72
Robert McGregor....	chestnut	bay	bay	42	1	1	30	2	1	77
Pilot Medium ..	gray	bay	gray	49	0	5	0	61	1	116
Jay Bird	roan	brown	roan	54	0	15	1	3	63	136
Margrave	roan	brown	roan	28	0	5	0	2	30	65

The mating together of blacks should produce only blacks and a few chestnuts, since chestnut is the only color hypostatic to black. However it does produce some browns and is recorded as producing occasional bays. The bays so far found are: Kipling 2:21¼, by Gambetta Wilkes ex Margaret W., and Gipsey Bel 2:30, by St. Bel ex Gipsey A. Now there is some reason to doubt the color of St. Bel. His dam was the black Beautiful Bells, but his sire was the bay Electioneer, and he is the only black among the eleven foals by Electioneer from black mares that I have found. Moreover, counting St. Bel, I have found only two black foals out of a total of 52 from Electioneer. Since Electioneer was homozygous for Hurst's factor this small proportion cannot be partly explained by supposing that more of the 52 would have been black had they had that factor. Neither is it possible to suppose that the small proportion is due to suppression by the gray or roan factor, since the 52 include only two grays and no roans. It looks very much as though Electioneer were homozygous for the bay factor. St. Bel died young, leaving few foals, so that I have been unable to get much data about his descendants. As to the other apparent exception, Kipling, I shall only call attention to the fact that neither he nor his dam are very well known. The following table shows foals from two black parents.

The case of foals from heterozygous sires and black mares is

Black Sire.	Foals from Black Mares.			
	Bays.	Blacks.	Browns.	Chestnuts.
Bellini	0	2	0	1
Gambetta Wilkes	1	3	0	0
Grattan	0	3	0	0
Patchen Wilkes	0	8	0	0
Sable Wilkes	0	7	1	0
Simmons	0	3	0	0
Nine others	1	8	3	0
Total	2	34	4	1

the one where the uncertainty about brown causes the most trouble. Since the presence of Hurst's factor is necessary before any of the three colors in question can appear I have left out the chestnut foals in the following table of foals from black mares.

Stallion.	Color	Foals from Black Mares.		
		Bays.	Blacks.	Browns.
Axtell	bay	1	4	3
Guy Wilkes	bay	1	7	3
Alcantara	brown	1	3	0
Allerton	brown	0	3	3
Nutwood	chestnut	2	3	0
Total		5	20	9

The numbers in the above table are small, and, as in the similar case with Hurst's factor, I have supplemented it with one giving all known foals, chestnuts being again left out.

Stallion.	Color	Foals				
		Bays.	Blacks.	Browns.	Grays.	Roans.
Bow Bells	bay	8	2	0	0	0
Directum Kelly	bay	7	2	2	0	0
McKinney	bay	14	4	8	0	2
Baron Wilkes	brown	44	10	28	0	1
Brown Hal	brown	34	10	15	2	4
Dictator	brown	12	7	5	0	0
George Wilkes	brown	12	5	3	0	1
Highwood	brown	24	5	8	0	0
American Star	chestnut	6	2	1	1	0
Mambrino King	chestnut	4	3	1	0	0
The Earl	chestnut	2	1	1	0	0
Conductor	gray	3	1	3	8	0
Pilot, Jr.	gray	3	2	1	10	0
Eagle Bird	roan	15	1	4	0	8

Now to turn to the gray factor. In the first place, I make no claims that all gray is epistatic to the four usual colors. Perhaps

it will be best to take up the grays by families, and I will first treat of those in which it is epistatic, and then of the one in which it seems not to be.

Most of the high-bred grays of to-day go back to Pilot, Jr., through an unbroken line of grays. This horse was a gray, son of a black sire and of a mare of untraced breeding whose color I have been unable to find. The gray sires in the next table all get their gray from him. This table includes all known foals except chestnuts, these being omitted for the same reason as in the last case. Since gray is an unpopular color it is safe to say that nearly all these foals were from recessive (*gg*) mares. I have so far found only one case of grays being mated together, and, since the produce of that mating was never heard from after racing, I know of no horse homozygous for the gray factor, *G*.

Gray Stallion.	Sire's Color.	Dam's Color.	Foals not Gray or Chestnut.	Gray Foals.
Bayard	gray	?	4	5
Pilot, Jr.	black	?	5	10
Pilot Medium	bay	gray	55	61
Re-election	bay	gray	12	15
Total			76	91

The following sires are all sons of gray members of the Pilot, Jr., family. Several of them have gray foals in this table, but all of these are from gray mares.

Stallion.	Color.	Foals				
		Bays.	Blacks.	Browns.	Grays.	Roans.
Lord Russell	bay	16	0	0	1	0
Peter the Great	bay	24	1	2	2	0
Darknight	black	5	2	2	0	0
Electricity	brown	10	1	7	1	0
Expedition	brown	46	3	10	5	0
Highwood	brown	24	5	8	0	0
Mambrino Russell	chestnut	6	0	3	0	0
Nutwood	chestnut	70	9	2	3	0

There can be little doubt that in the Pilot, Jr., family gray is an ordinary dominant, and there are other families where it seems to be, though there is not as much evidence. One of these goes back to the mare Sontag Mohawk, and through her probably to imported Messenger, the foundation of the breed of American

harness horses. Another goes to the mare Bashaw Belle, daughter of Young Bashaw, gray. Of the three horses below, Conductor is a son of Sontag Mohawk. Manager is a grandson of Bashaw Belle, and Aleryon is out of Lady Blanche, daughter of Privateer, both grays.

Gray Stallion.	Sire's Color.	Dam's Color.	Foals not Gray or Chestnut.	Gray Foals.
Aleryon	bay	gray	14	7
Conductor	bay	gray	7	8
Manager	chestnut	gray	11	12
Total			32	27

Eros and Walnut Hall, a brother and son, respectively, of Conductor, and both browns, have no gray foals among the 35 I have found.

There is one family in which gray appears not to be epistatic. The first member of the family that I know of is General Wilkes, gray, son of George Wilkes (an ordinary brown which appears in two of the tables already given and has nine different sons in them) and of a gray mare. This stallion had some gray foals, of whose descendants I have found no record. But he had two sons not gray which have produced many gray foals. They are Dispute, black, and Bobby Burns, bay. I am not sure of the color of the dams of any of Dipute's gray foals, but in the case of Bobby Burns some of them are from bay mares. Dispute's maternal color pedigree I do not know, but Bobby Burns is from Dixie, a bay daughter of the brown Dictator appearing in some of the first tables in this paper. The colors of all foals found from Dispute and Bobby Burns are:

Dispute: bay, 3; black, 2; gray, 4; all, 9.

Bobby Burns: bay, 40; black, 10; brown, 10; chestnut, 3; gray, 43; all, 106.

This is certainly a different kind of gray from the others just described, but I have not enough data to try to explain it.[1]

[1]In connection with the bay and gray factors I may quote the following from the pen of Professor Karl Pearson ("The Law of Ancestral Heredity," *Biometrika*, 1903, vol. 2, p. 214), though it was written about the English thoroughbred: "If black or gray coat-colour in horses were 'recessive,' when two blacks were mated we should expect only black offspring, but black can disappear for a generation or even two and then reappear. Or, take a case like that of a gray horse Viscount,

The last character I have to deal with is roan. This, like gray, is epistatic to the four usual colors in most families, but may not be in all.

Many of the roans of to-day go back to the old roan race-mare Lady Franklin, through her daughter Lady Frank and grandson Jay Bird, both roans. Jay Bird sired Eagle Bird, Jay Hawker, Allerton and Jackdaw, and Jay Hawker sired Jay McGregor. The following table shows all foals but chestnuts.

Stallion.	Color.	Foals.					
		Bays.	Blacks.	Browns	Grays.	Roans.	All not Roan.
Eagle Bird	roan	15	1	4	0	8	20
Jay Bird	roan	54	0	15	3	63	72
Jay Hawker	roan	6	0	0	0	13	6
Total		75	1	19	3	84	98
Jay McGregor	bay	11	1	5	0	0	17
Allerton	brown	32	8	20	2	1	62
Jackdaw	brown	9	3	12	1	0	25

Allerton's roan foal is from a roan daughter of Jay Bird.

Another family goes back to Laura Fair, roan, through her roan granddaughter Spanish Maiden. This last mare produced 1 bay and 3 roans, including the sire Margrave. Tom Hal, Jr., founded another family of roans, and another goes to the roan mare Tilla, which had 4 bay foals, 1 brown and 3 roans, the latter including Fred S. Wilkes. The Brown Hal appearing in the table below is a son of Tom Hal, Jr. Chestnut foals are omitted as before.

Stallion.	Color.	Foals.	
		Not Chestnut or Roan.	Roan.
Fred S. Wilkes	roan	13	8
Margrave	roan	35	30
Tom Hal, Jr.	roan	7	7
Total		55	45
Brown Hal	brown	61	4

where gray remained dominant for three generations only to disappear before the chestnut of the mare Blue Stocking in the Viscount and Blue Stocking filly Miss Johanna!" Just what that passage was intended to mean is a problem which I have not yet solved. What do blacks produce when mated together, and what has that to do with skipping a generation or so? And if a recessive cannot skip what can? Certainly not a dominant. What is to prevent us from supposing Viscount a heterozygote?

The roan and also the two gray foals of Brown Hal probably get their color from their dams, since in all cases these were daughters of roan or gray sires. As to the kind of roans these are I can only say that Margrave and one of his foals, and one of the foals of Tom Hal, Jr., are all red roans.

I have found six roan foals which had neither parent roan. One was from a dun mare. One was from two bays and another was from a chestnut and a bay, the chestnut being Robert McGregor, which almost certainly carried no gray factor. Another was from a bay sire and a chestnut dam. The other two each had a gray parent, and at least one of them was a black roan. Now only six roans produced by the thousands of cases of mating together horses not roan is a very small percentage. It is to be noticed that these six are not closely related. Even the two grays concerned are one Pilot, Jr., and one Sontag Mohawk. It seems to me probable that red roan at least is an ordinary dominant, and that all but one of the above cases are mistakes or exceptions. It is quite possible that all horses having the factor *R* are roan, the type of roan depending upon the color the horse would have been if he had not had that factor. This, if correct, explains away the difficulty presented in the next paragraph in so far as roan is concerned.

The relation between gray and roan is not clear. It seems probable that some of the *black* roans may be connected with gray, but an examination of the tables given above will convince one that, in general, the two colors are quite distinct. I have found only two instances of the mating together of grays and roans, and in both the result was gray. Of course much more evidence would be needed in order to find out how they act toward each other. The relation between these two factors and the bay factor is also not quite clear. It is evident that the presence of either can conceal bay, but whether or not either can appear in the absence of the bay factor is not certain. I am inclined to think that they can. If gray cannot we have an explanation of the gray foals from the black Dispute, but get no help in the much harder problem concerning his bay half-brother, Bobby Burns. One would expect to find some chestnuts carrying gray or roan factors (if the *ChhR* horses are chest-

nut roans there should be no chestnuts carrying the roan factor). With one possible exception in the case of each color I have found no such case. How unsatisfactory these two cases are will appear from the color pedigrees of the horses concerned.

Molly Morton, gray.	Banker Rothschild, brown.	Rothschild.
		Pilot Anna, gray.
	Lady Forrester, chestnut.	Royal George, chestnut.
		Belle of Saratoga, brown.
North Wind, roan.	Young Clay Pilot, bay.	Clay Pilot, bay.
		?
	Lola M., chestnut.	Roy Executor, brown.
		Light Dale, chestnut.

Summary.

This study of the pedigrees of blooded trotters indicates that the color of such horses is usually controlled by five factors, as follows. First, a factor for chestnut, *C*, present in all the horses studied. Second, a factor for black, Hurst's factor, *H*, epistatic to the factor *C*, and hypostatic to the three following. Third, a factor for bay, *B*. Fourth, a factor for roan, *R*. Fifth, a factor for gray, *G*. *R* or *G* inhibits *B* if it is present, but whether they depend upon its presence for their own appearance or not is not clear.

Columbia University,
June, 1910.

THE MARSUPIUM OF THE ANODONTINÆ.

In the June number of the BIOLOGICAL BULLETIN (p. 31), G. Lefevre and W. C. Curtis have published a paper on "the Marsupium of the Unionidæ," in which they say that the lateral (secondary) water tubes cut off from the original (primary) water tubes in the marsupium of the Anodontinæ, described by myself in *Nautilus*, February, 1910, are *not present*. In order to show this, they publish three figures of horizontal sections through the marsupia of three species of Anodontinæ.

To obviate misunderstanding, I want to point out, that, in two of the figures referred to, these secondary water tubes ARE PRESENT, most *beautifully* and *typically* in Fig. 1. In Fig. 3 traces of them are observable, while in Fig. 2 they are *not yet* developed. These tubes are *not* blood vessels, as might be believed after superficial investigation.

For the rest, I cannot go into detail, but must refer to my future publication (illustrated by microphotographs) in the *Memoirs of the Carnegie Museum*, where additional facts will be published.

A. E. ORTMANN.

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ACCESSORY CHROMOSOMES IN MAN.

MICHAEL F. GUYER,

UNIVERSITY OF CINCINNATI.

Since the publication of my papers on the spermatogenesis of the guinea and of the chicken respectively (Guyer '09*a*, '09*b*) in which was recorded the finding of a chromosome or chromosome complex comparable to the "odd," "accessory" or "X-element," described so frequently of late as occurring in a wide range of the Arthropoda, particularly the Tracheata, I have examined material from other vertebrates and can at present record its presence in the rat, its probable occurrence in the pigeon (although this will require some further corroboration), and its conspicuous occurrence in man. Inasmuch as the material for its study in the rat is in the hands of a student for further investigation, I shall confine myself in this paper to a description of the two accessory chromosomes as found in man, together with other features of human spermatogenesis.

For my studies on man I have been fortunate in being able to obtain exceptionally good material through the courtesy of my colleague, Dr. Paul G. Woolley. The subject from which the testicular material was secured was a negro thirty years of age who had died suddenly from the rupture of an aortic aneurism (Case no. 15643, Pathological Records, the Cincinnati City Hospital). A testis was removed within between an hour and an hour and a half after death while the body was still warm and slices were placed immediately into Gilson's and into Bouin's fluids.

The mounted sections were from five to twelve microns thick. Most of them were stained in Heidenhain's iron-hæmatoxylin

and counter-stained with Congo red or acid fuchsin, although Delafield's hæmatoxylin was used with some.

An abundance of cell divisions were found to have been in progress at the time of death. In a given field of the microscope, in a favorable region, it was not unusual to observe as high as six or seven cells in various phases of division. As many as five or six of such areas might exist in a single section, although it was more usual to find only one or two. The material was very uneven in that slides would be found in which section after section showed division stages, while in others divisions were scarce. These facts indicate that there were proliferating and resting zones in the testis. The stages found in most abundance were the metaphases and late prophases of the primary spermatocytes. It was a comparatively simple matter to find spindles on which the ordinary chromosomes were in metaphase with the two accessories, closely associated, well removed toward, or at, one pole (Figs. 6, 7, 8 and 9).

In the literature of the subject much confusion prevails regarding the number of chromosomes characteristic of man. There is wide disagreement in the counts of different observers and there seems to have been a great dearth of material showing division stages. Most of the enumerations are based on observations of from two to eighteen cells and these often in questionable stages of preservation. The great difficulty apparently has been to secure material which was sufficiently fresh or which was not diseased tissue that is notoriously irregular as regards karyokinetic phenomena.

As early as 1881 Flemming discussed mitosis in the case of man illustrating it with some six figures (Taf. 3, Figs. 11–16) of which Fig. 16 is from leucocytes of leucemic blood, the others, from the corneal epithelium of two different subjects from each of whom an eye had been removed because of affection of the bulbus. Although at this time he made no definite record of the number of chromosomes, his drawings show them to be considerably in excess of sixteen, the number later announced by Bardeleben ('92).

Writing several years later, however, in response to the 1892 paper of Bardeleben, Flemming ('97), from a reëxamination of

his old material, gives the number of chromosomes as twenty-four. He cites the papers of Hansemann ('91, '93) as the earliest attempts known to him to make a count of the chromosomes of man. But since Hansemann records eighteen in one case, twenty-four in another, and forty in a third, the latter apparently estimated from the spireme stage, and inasmuch as he himself admits that his count was very uncertain, concluding with the statement that, "die Zahl sicher höher als 24 sei," we may fairly disregard it, I think, in the light of modern technique. In this second paper Flemming ('97) states that his count is based on only four cell-divisions in which the chromosomes had just split preparatory to separation. His exact statement of his observations is as follows: "Es gelang das zwar bei keinen ganz sicher, aber bei zweien der vier darin enthaltenen Mitosen doch annähernd; es scheinen in beiden Fällen 24 Doppelchromosomen zu sein. Bei beiden sind es jedenfalls mehr als 22 und, wie ich sagen zu können glaube, weniger als 28; an einiger Stellen decken sie sich so, dasz eine exacte Zahlung mir unmöglich wird." Flemming's material had been fixed in one sixth per cent. chromic acid and stained with safranin.

Bardeleben has published three papers ('02, '07, '08) on the spermatogenesis of mammals including man in which he comes to the conclusion that the number of chromosomes in the spermatogonia and spermatocytes of man are sixteen and eight respectively, and in his later papers he sets down four as the number that ultimately reaches the spermatids. That is, there is in successive divisions a reduction in numbers from sixteen to eight and then from eight to four. This is much the condition that I have found prevailing in birds (Guyer, '02, '09).

Wilcox ('00) studied sections from a testis which had been removed from a man fifty-four years old, in an operation for hernia. Although scrotal swelling had existed for a year previous to the operation the testis seemed to be normal in size and appearance. He reports that, "In my material the number seemed to be eighteen, the different counts resulting in figures ranging from fifteen to nineteen." He remarks however upon the striking absence of karyokinetic stages, so that his observations were based upon a very limited number of divisions. Because of

this lack of favorable stages he says: "The whole organ was, therefore, sectioned and in the great number of sections thus obtained, not more than twenty cells were found in mitotic condition."

Wilcox continues: "The few cases of mitosis observed were in spermatocytes of the first order. One could easily distinguish spermatogonia, spermatocytes of the first and second order, spermatids, and numerous nearly mature spermatozoa. The number of the latter to be seen was very large and precludes the assumption that the testis was functionally impaired by age or by hernia. In the opinion of the writer, this condition merely indicates that all the various processes in the spermatogenetic series are not necessarily to be observed as taking place at the same time. I can see no reason why there might not become established in the testis periods of cellular activity alternating with periods of cellular rest."

Unfortunately Wilcox gives no drawings with his paper nor does he state definitely whether he regards the eighteen chromosomes seen in the spermatocytes of the first order as the reduced number or not. He does remark, however, that, "in many cases they were plainly arranged in the tetrad or ring formation which has been observed in a pretty general variety of investigated species," consequently the inference would be that a synapsis had occurred and that one might expect to find in the neighborhood of thirty-six as the somatic number.

The latest investigation on the number of chromosomes in man with which I am acquainted is that of Duesberg ('06). He reviews the work of Hansemann, von Bardeleben, and Flemming and on the strength of his own observations concludes that Flemming's count of twenty-four is correct. The excessive number found by Hansemann he would account for on the basis of the abnormal increase in the number of chromosomes which is likely to occur in pathological tissues. In the case of Bardeleben he is inclined to believe that very thin sections (three microns) are responsible for the smallness of the count since he regards it as probable that part of the cell had been cut away.

The tissue upon which Duesberg worked had been fixed in Flemming's or in Hermann's fluid and stained by the iron-hæ-

matoxylin method. However, the number, twenty-four, which he records for man was not determined by direct count but was inferred from the fact that he found two or three clear cases of twelve chromosomes in the primary spermatocytes. That his finding of twelve in the primary spermatocytes was correct is borne out by my observations but he is not justified, in consequence, in stating that there must be twenty-four in spermatogonial or somatic cell-divisions. My material shows that two of the twelve chromosomes are the univalent accessories, and a clear count of favorable spermatogonial chromosomes reveals only twenty-two. This means in all probability that of the twelve chromosomes of the primary spermatocyte, ten are bivalent and two accessories. Although Duesberg examined the spermatogonial chromosomes he states that he was unable to count them exactly beyond determining that, contrary to the opinion of Von Bardeleben, there were clearly more than sixteen. He states further (p. 477) that, "Je n'ai pas pu les compter exactement, tant à cause de la petitesse des cellules que du nombre assez élevé des chromosomes, mais dans quelques cas favorables où leur numération a pu être entreprise, j'ai obtenu des résultats très voisins de 24, jamais supérieurs à ce nombre dans les cellules normales." And in conclusion he says: "Il résulte de là que le nombre des chromosomes est certainement à mon avis, de 12 dans les spermatocytes et par conséquent de 24 dans les spermatogonies et les cellules somatiques. C'est la confirmation de l'opinion de Flemming."

In the general scheme of the spermatogenesis of man there appears to be nothing unique. One can readily recognize the usual four generations of germ cells; viz., spermatogonia, primary spermatocytes (or spermatocytes of the first order), secondary spermatocytes (or spermatocytes of the second order), and lastly spermatids which transform directly into the spermatozoa. An abundance of easily identified Sertoli or nurse cells are in evidence. Occasional centrosomes were observed in suitably stained preparations but I have not pictured any in my drawings because the preparations from which the latter were made were all so strongly decolorized that the stain had evidently completely disappeared from any centrosomes which might have been present.

The matter of counting the spermatogonial chromosomes, it must be admitted, is one of great difficulty. In the late prophase or equatorial plate stage, the only time at which a count is possible, they lie for the most part in an irregular band around a central clearer area. In the vast majority of cases only a deeply stained mass of small contiguous or overlapping chromosomes is visible in this band and an accurate count is out of the question although one can frequently determine that there are over twenty. In several instances, however, in which the positions of the chromosomes and the degree of the staining were favorable, twenty-two distinct chromosomes, never more, were visible.

There is considerable range in size among the individual chromosomes of the spermatogonia as well as observable differences of form. Most of them were rod-like or oval in shape although some were more nearly spherical. In several though by no means all instances two chromosomes, closely associated, were seen lying at some distance away from the main band, out in the cytoplasm. Taking into account this isolation, the rounded shape of these chromosomes and their relative sizes, it seems very probable that they are the two accessory chromosomes which do not manifest their presence for a certainty until the next division. It will be observed that one is somewhat smaller than the other. This condition obtains also between the two chromatin nucleoli of subsequent stages as well as between the accessory chromosomes wherever they can be identified, and one is led in consequence to strongly suspect that they are all one and the same thing. This inference is all the more justifiable when the relation between the chromatin nucleoli and the accessory chromosomes in some of the lower forms is recalled.

Fig. 2 represents a nucleus of the primary spermatocyte in the spireme stage which shows the two chromatin nucleoli in question. In deeply stained specimens these nucleoli, especially the smaller one, are not always evident but in preparations stained by the iron-hæmatoxylin method and then almost entirely decolorized, even as regards the ordinary chromatin of the spireme they are usually conspicuously visible. It should be mentioned that occasionally other small nucleolus-like granules were observable but since there was no constancy in their presence, size or relationship, I have

felt justified in ignoring them in the present discussion. Fig. 3 represents the spireme in the contraction phase which is not very pronounced in man. It will be observed that the two characteristic chromatin nucleoli still persist.

The primary spermatocytes when ready for division, as has already been stated, reveal twelve chromosomes in late prophase or early metaphase (Figs. 4, 5). In Fig. 4 the two accessories are seen at a glance and the remaining chromosomes, judging from their increased size and changed form, are bivalent, representing the paired univalent chromosomes of the spermatogonium. That is, of the original twenty-two chromosomes twenty have paired to form the ten bivalents of the primary spermatocyte and two have remained unpaired as the accessory chromosomes. In Fig. 4 it is not evident just which two are the accessories although twelve chromosomes are present.

It is obvious from the the figures (Figs. 4–9) that there is considerable difference in the size of the various chromosomes of the primary spermatocyte. Although the attempt was made it was not found possible to always identify the individual chromosomes. They grade down in size from some three or four large ones to two or three small ones but the fluctuations in size, probably due for the most part to differences in the effects of fixation together with different degrees of extraction of the stain, were too great to render identification sure. In very strongly decolorized sections, especially when counterstained with Congo red, one large chromosome in particular frequently exhibited a tetrad-like formation, while the other large ones at times showed more or less definite indications of lobing. In some cases this was sufficiently marked to interfere with accurate counting. In a very few instances, so few I think as to be practically negligible, there appeared to be fourteen instead of the customary twelve chromosomes, but the extra chromosomes always took the form of a tiny pair which I am inclined to think had become split off from one of the ordinary tetrads or which had through some chance never entered into the proper tetrad formation. They were always united by linin-like strands to one or two of the larger chromosomes.

Figs. 6, 7, 8 and 9 show the two accessories in characteristic positions. Side by side, they always pass entire, considerably in advance of the divided ordinary chromosomes, toward one pole.

Of the ordinary daughter chromosomes of this first spermatocytic division, a pair of small elongated ones not infrequently are the first to emerge from the general equatorial mass as shown in Fig. 7. One is led to suspect that they may possibly be comparable to the small pair of chromosomes found so constantly in certain of the Tracheata although the evidence is not sufficiently decisive to make this an established fact.

It is inferred that the division of the primary spermatocyte is the reducing division, not simply because such a division ordinarily occurs at this stage, but from the fact that the chromosomes after divergence (Figs. 10, 11) when compared with corresponding divisions of the secondary spermatocytes are seen to resume more the elongate, rod-like appearance that characterizes the univalent spermatogonial chromosomes, and also because the accessory chromosomes pass over entire to one pole here while they are halved in the next division.

It is evident from the foregoing that as regards chromatin content the result of the division of the primary spermatocyte is the production of two dissimilar cells, one of which receives ten, the other, twelve chromosomes. Fig. 10 is a drawing of one end of a late anaphase of such a division showing twelve chromosomes (10 plus 2 accessory). Fig. 11, in which only ten chromosomes are visible, was drawn from what is probably the reverse end of a somewhat later anaphase than that shown in Fig. 10. It is just possible that it is a prophase of division in a secondary spermatocyte where univalent chromosomes come to the equator, but if so it is the exception rather than the rule, as the secondary spermatocytes ordinarily divide according to a different scheme. In any event the drawing serves to illustrate the fact that some daughter cells of the primary spermatocytes have twelve chromosomes and some only ten.

In places both primary and secondary spermatocytes were found dividing in the same field and one is led to conclude that either there was no intervening period of rest between the two divisions or that it was a very brief one. In other instances, however, undoubted resting stages of secondary spermatocyte nuclei were seen in abundance. Approximately half of them showed, under proper decolorization, two chromatin nucleoli of which one was somewhat smaller than the other.

While at the conclusion of the divisions of the primary spermatocytes ten and twelve chromosomes respectively were delivered to the pairs of daughter cells, nevertheless, when the latter as secondary spermatocytes become ready for division, half of them show five and the remainder seven chromosomes. A second pairing of the ordinary chromosomes has evidently occurred, so that there are five bivalent chromosomes in each type of cell and the additional two accessories in the one type. Fig. 12 is a drawing of two contiguous secondary spermatocytes; the one shows five bivalent chromosomes in late prophase, the other more than five chromosomes in metaphase. These two cells are undoubtedly the two daughter cells of the same primary spermatocyte. Fig. 13 shows one daughter cell containing a group of seven chromosomes and the other a late anaphase of division which shows at one end five chromosomes. The number of chromosomes at the opposite pole of the second cell should of course be five although because of the dense massing it could not be positively determined. Fig. 14 represents a late anaphase of division in a secondary spermatocyte which manifestly had had seven chromosomes in metaphase.

Both accessory chromosomes divide in this second spermatocytic division period so that each resulting spermatid receives seven chromosomes (Fig. 10). Fig. 15 represents an anaphase of division in a secondary spermatocyte showing still at the equator of the spindle a lagging chromatic mass. Such a condition was found in several instances and while I believe it to be the two accessory chromosomes which happened merely to be unfavorably placed for observation, I could not positively identify it as such. From the relative positions of the chromosomes as seen in Fig. 16 one would infer that the two sets of accessories were the last to have passed from the equator to the poles of the spindle. Moreover, such a lagging of the accessory in this division was observed in both the guinea and the chicken (Guyer, '09).

It should be mentioned that occasional division stages were visible which, judging from the smallness of the cell and the size and shape of the chromosomes, looked as if they might be secondary spermatocytes preparing to divide with the univalent type (ten or twelve) of chromosome. It is possible, for instance, that

Fig. 11 represents a prophase of the secondary rather than an anaphase of the primary division although I am inclined to think it is the latter. If such simple divisions do take place, however, they are certainly scarce in the material which I have examined so far.

From the foregoing evidence it is manifest that there are in all two distinct groups of spermatids equal in number; namely, those which have received five and those which have received seven chromosomes. These chromosomes soon lose their visible identity and the spermatids are apparently all alike except for the significant fact that approximately half of them, in such preparations as have been stained by the iron-hæmatoxylin method and then all but entirely decolorized show two chromatin nucleoli. It would seem probable that these nucleoli stand in direct genetic continuity with the two eccentric chromosomes seen in the spermatogonia and the two chromatin nucleoli and the accessory chromosomes of the spermatocytes. Fig. 18 represents two contiguous spermatids, one of which shows no nucleoli, the other, two. Comparison with Fig. 19 shows the relative conditions of size between the nucleoli of the spermatid and those of a primary spermatocyte.

As to the meaning of the second conjugation there seems to be at present no clew. I have commented on it briefly in a former paper ('09*a*, p. 509). It is not peculiar to man for I have observed it also in the pigeon ('02, '03), the guinea ('09*a*) and the rooster ('09*b*). Undoubtedly Bardeleben ('97, '98) still earlier saw the same phenomenon in man, for although my results do not agree numerically with his count of sixteen, eight and four respectively, evidently, from the relative proportions in his counts, he had come upon this second curious numerical reduction.

Assuming that the respective chromosomes are more or less qualitatively differentiated, such a numerical reduction, however, by no means necessarily implies that there has also been a second qualitative reduction. Aside from the improbability of such a reduction, the general appearance of the divided chromosomes would not warrant this interpretation; for instead of the elongated univalent type as seen in the spermatogonia or in anaphases of the divisions of spermatocytes of the first order, the daughter chromosomes here retain the rounded appearance and increased size that

is characteristic of the bivalent types (compare Figs. 1, 10 and 11, 13, 14, 15, 16 and 17). Thus while half of the spermatids receive five, and half seven chromosomes, in terms of univalence the numbers would in all probability be ten and twelve respectively.

Inasmuch as the spermatids transform directly into spermatozoa it follows that there must be be two classes of the latter differing with respect to whether they have or do not have the two accessory chromosomes. Thus the conditions in man appear to be very much the same that Wilson ('09) describes for *Syromastes*, one of the squash-bug family (Coreidæ).

Numerous examples of such dimorphism of the spermatozoa have been recorded in various invertebrates, particularly in insects, arachnids and myriapods, and it has been clearly demonstrated that eggs fertilized by spermatozoa which possess this accessory chromosome or chromosome group (there may be one, two, three or even four separate chromatic bodies, depending upon the species) develop into females, those fertilized by spermatozoa which do not possess it, develop into males. Hence the accessory has come to be regarded by some of our most careful and experienced workers as an actual sex determinant. In any event it is obviously associated with the determination of sex either as cause or effect. In the light of numerous recent researches both on plants and animals the idea has rapidly gained ground that sex arises not as was long believed, as a response of the developing organism to stimuli from without, but that under normal conditions at least, it is automatically determined by some internal physiological mechanism.

Inasmuch as this intricate matter has been repeatedly and exhaustively discussed pro and con during the past ten years, it is unnecessary for me to enter into a review of the subject anew. For the general reader who may not have kept in touch with the current literature of the subject, two excellent critiques are now available in the recent papers of Wilson ('09*a*) and Morgan ('10). In these papers one will also find thoroughgoing discussions of the subtle problem as to whether, assuming that the accessories are sex determinants, the matter of sex determination is to be regarded as a qualitative process effected by some inherent peculiarity of the accessory chromosome, or whether the relation of such a chro-

mosome or group of chromosomes to sex is merely a quantitative one, the female type resulting when a greater amount of active chromatin is present. Extensive bibliographies will be found in the recent papers of Wilson ('05, '06, '09), Payne ('09), Morse ('09) and Morgan ('10).

In conclusion I wish merely to point out that as regards accessory chromosomes, conditions prevail among vertebrates (guinea, chicken, rat, man, etc.) similar to those found among numerous Tracheata (and probably certain other invertebrates) where the accessories are undoubtedly associated in some way with the phenomena of sexuality. In *Syromastes* (Wilson, '09*b*), which seems to parallel most nearly the condition found in man, half of the spermatids were found to possess two more chromosomes than the remainder. It was predicted by Wilson that in consequence the somatic cells of the female of this species would show two more chromosomes than the somatic cells of the male. Later the facts were found to be in exact accord with his prediction, the somatic cells of the female containing twenty-four, of the male twenty-two chromosomes. Similar verifications have been made in other tracheate forms.

In the light of these facts we should expect the somatic cells of man to contain twenty-two, and of woman, twenty-four chromosomes. The tissues of the female have not yet been studied with this in mind. Flemming ('97) records the somatic number of chromosomes, determined from corneal cells, as twenty-four but unfortunately he does not record the sex of the subjects from which the material was obtained. If it were a female his count would bear out the interpretation given above.

Summary.

1. Twenty-two chromosomes differing considerably in size occur in all spermatogonia in which a definite count could be made. In a few instances two, apparently the two accessory chromosomes, were seen considerably to one side of the main mass of chromosomes, surrounded by a small clear court of cytoplasm.

2. Twelve chromosomes appear for division in the primary spermatocyte, of which ten are evidently bivalent and two accessories.

3. The two accessory chromosomes pass undivided to one pole of the spindle considerably in advance of the other chromosomes, with the result that half of the daughter cells in this division receive twelve, and half, only ten univalent chromosomes. This is evidently the reduction division.

4. The ten univalent chromosomes which passed to the one secondary spermatocyte unite again in pairs, at least in the majority of cases, to form five bivalent chromosomes which appear at the equator of the spindle when the cell is ready for division. The division here is presumably an equation and not a second reduction division, judging from the size, shape and general appearance of the resulting daughter chromosomes. Thus while each of the spermatids formed as a result of this division receive only five chomosomes, the latter are bivalent and equivalent to ten of the somatic or spermatogonial chromosomes. There is some slight evidence that the secondary spermatocytes may occasionally divide with these chromosomes in their original condition of univalence.

5. Ten of the twelve chromosomes which passed to the other pole of the spindle in the primary spermatocyte behave in precisely the same way as described in the last paragraph. The two accessory chromosomes come to the equator of the spindle in the secondary spermatocyte with the five bivalents thus making in all seven. Each accessory now divides so that the resulting spermatids each receive seven chromosomes; that is, five bivalent plus two accessory, or the equivalent of twelve univalent chromosomes.

6. In reality, then, of the total number of spermatids, half have in all probability received ten, and half, twelve (10 plus 2) univalent chromosomes. Inasmuch as the spermatids transform directly into spermatozoa, there must be two classes of the latter differing with respect to whether they have or do not have the two accessory chromosomes.

7. It is a significant fact that approximately half the resting spermatids when strongly decolorized after iron-hæmatoxylin staining, show two chromatin nucleoli and half do not. It seems probable that these nucleoli may correspond to the accessory chromosomes and are to be identified with the two

nucleoli of the primary spermatocyte and the two eccentric chromosomes seen in the spermatogonia.

8. It is probable that in man and certain other vertebrates, as in the insects, myriapods and arachnids, the accessory chromosomes are in some way associated with the determination of sex.

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Explanation of Plate I.

All of the drawings were made with the aid of a camera lucida; their magnification is approximately 1,550 diameters. While the chromosomes are represented as accurately as possible, no attempt has been made to show details of the achromatic structures beyond general appearances and relations. In several cases where the chromosomes were not in the same focal plane they have been drawn carefully in the most favorable plane and then corrected as much as possible from second more accurate drawings of such individual chromosomes as were not in focus in the first drawing.

Fig. 1. Late prophase of spermatogonial division showing twenty-two chromosomes. The two chromosomes lying to one side of the main zone of chromosomes are presumably the two accessories.

Fig. 2. Nucleus of primary spermatocyte showing spireme stage with two chromatin nucleoli.

Fig. 3. Nucleus of primary spermatocyte showing the contraction-phase of the nuclear contents, also two persisting nucleoli.

Fig. 4. Late prophase of division in a primary spermatocyte showing twelve chromosomes. The two lying to one side of the main group are the accessories.

Fig. 5. Late prophase of division in a primary spermatocyte showing twelve chromosomes. Here the two accessories are not readily identified.

Figs. 6, 7, 8, 9. Metaphases of divisions in primary spermatocytes showing the two accessories in characteristic positions passing early to the poles. Fig. 7 shows also two precociously diverging daughter chromosomes.

Fig. 10. One end of a late anaphase of division in a primary spermatocyte showing twelve chromosomes (10 plus 2 accessory).

Fig. 11. Probably one end of a late anaphase of division in a primary spermatocyte, showing ten chromosomes, the accessory chromosomes having gone to the opposite pole; possibly a prophase of division in a secondary spermatocyte in which the ten chromosomes have remained univalent.

Fig. 12. Two contiguous secondary spermatocytes of which one shows five bivalent chromosomes in late prophase, the other more than five chromosomes (probably five bivalent plus two accessory) in metaphase. These two cells are evidently the products of a division of a primary spermatocyte in which ten chromosomes passed to one pole and ten plus the two accessories to the other.

Fig. 13. Two contiguous secondary spermatocytes. One, having just divided, shows five chromosomes at one pole; the chromosomes at the other pole are so massed as to preclude counting although there should be five. The other secondary spermatocyte shows seven chromosomes in late prophase.

Fig. 14. Late anaphase of division in a secondary spermatocyte which has received the two accessory chromosomes, showing seven chromosomes in all.

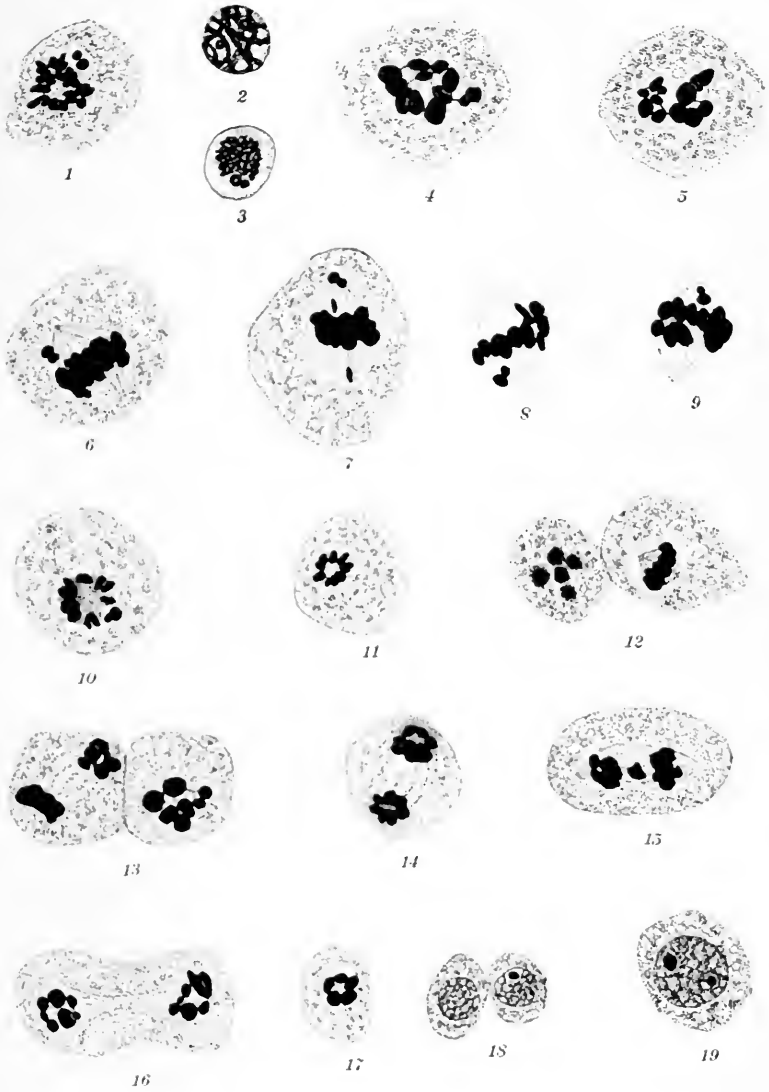
Fig. 15. Anaphase of division in a secondary spermatocyte showing still at the equator a lagging chromatic mass which is probably the two accessory chromosomes although it could not be positively identified as such.

FIG. 16. Late anaphase of division in a secondary spermatocyte which has received the two accessory chromosomes. Each of the latter divides as an independent chromosome at this time.

FIG. 17. One end of a late anaphase of a division in a secondary spermatocyte which had not received the accessory chromosomes.

FIG. 18. Two contiguous spermatids, one without chromatin nucleoli, the other with two. The spermatids in general are about equally divided into these two classes.

FIG. 19. Nucleus of a primary spermatocyte showing two chromatin nucleoli.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ON THE RIGHTING MOVEMENTS OF THE STARFISH.[1]

A. R. MOORE.

In an article entitled "The Behavior of the Starfish, *Asterias Forreri* de Lariol,"[2] Jennings discusses the movements of that animal in righting itself. He assumes that the starfish makes its movements in order to adapt itself to its environment and that therefore these movements are purposeful. From this assumption he concludes that "when the starfish is turned over on its dorsal surface locomotion is impossible, the finding and capture of food must stop; the delicate gills are pressed against the bottom, injuring them and impeding respiration; and displacements of the internal organs must occur that may be harmful to their proper functioning. We find, as might be anticipated, that there is a regulation of these bad effects by movement; the starfish turns again on its ventral surface."

A much simpler explanation of the righting movements has been given by Loeb.[3] He points out that the tube feet are positively stereotropic. Therefore the arms twist and turn until all of the tube feet are in a position to be in contact with a surface.

I have made observations on about thirty specimens of *Asterina miniata* and a like number of *Asterias ochracea*, with a view to determining the nature of the righting movements.

Let us look first at the causes, which Jennings has given us, for the starfish righting itself.

In regard to the first, viz., that locomotion is one of the ends which a starfish has in view in righting itself, I have found that very frequently the starfish crawls up the side of the aquarium and, upon reaching the surface of the water, thrusts out three or four arms dorsal side downward, their tube feet clinging to the surface film of the water. In such a position they remained

[1]From the Herzstein Research Laboratory, New Monterey, Cal.

[2]Jennings, *University of California Publications*, Zoology, Vol. 4, pp. 53–185.

[3]Loeb, "Comparative Physiology of the Brain," Chapter 3.

sometimes for more than an hour, although further locomotion was impossible, and no attempt at righting was made. In fact, the starfish often retained such a slight attachment to the wall of the aquarium that the surface film of the water could no longer support the weight of the animal, with the consequence that the latter fell to the bottom of the tank. In such cases the tube feet cling to the surface film of the water because the film acts as a solid surface; it cannot, however, bear the animal's weight. Romanes[1] speaks of these movements as follows: "On reaching the surface, the animal does not wish (!) to leave its native element . . . and neither does it wish (!) again to descend into the levels from which it has just ascended. It therefore begins to feel about for rocks or sea weeds at the surface, by crawling along the side of the tank and every now and then throwing back its uppermost ray or rays along the surface of the water to feel for any solid support that may be within reach." Romanes evidently was not familiar with surface tension. Had he known that the surface film of a liquid acts like a solid surface he would have been prevented from attributing intelligence to the starfish.

In order to see whether pressure on the gills might, as Jennings states, cause the starfish to turn over, I supported a glass plate in the aquarium, at a height just sufficient to press lightly on the dorsal side of a starfish moving over the floor of the tank. This was placed in the path of an approaching starfish. The latter did not change its direction when the plate was touched, but pursued its course, although the gills were pressed down. Furthermore, if a starfish is allowed to attach itself to a glass plate and is then suspended dorsal side downward so that it touches the bottom, its movements continue normally, although it could easily right itself if that were necessary. Clearly, then, pressure on the gills is not one of the factors which causes a starfish to right itself.

The displacements of the internal organs which, we are assured, "must occur" when the dorsal side is down, can only be due to gravity. I have frequently observed large numbers of starfish clinging, dorsal side downward, to overhanging ledges, feeding

[1]Romanes, "Jellyfish, Starfish and Sea Urchins," p. 268.

on barnacles and mollusks. Surely, "the displacements of the internal organs which *must* occur" when the dorsal side is downward, do not interfere in the least with the ingestion and digestion of food. Such "displacements" can, therefore, hardly be considered seriously as causes for the righting movements taking place.

We are forced to conclude, from the observations described, that, as Loeb has stated, the starfish ceases its efforts to right itself the moment all the tube feet can be brought into contact with a solid surface. Gravity plays no part in the righting movements.

The idea has been advanced by Loeb[1] that the mechanism of the righting movements is the result of coördinating and inhibiting impulses, which are transmitted to the various arms by the ventral nerve ring.

Six distinct methods of the righting reaction have been described by Jennings, but he has made no analysis of them on the basis of inhibiting and coördinating impulses. My observations agree with Loeb's assumption and give a rather simpler explanation of the behavior of the starfish.

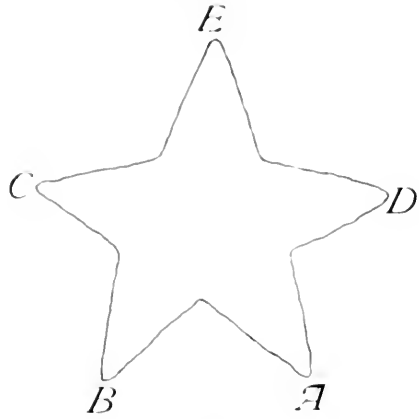

FIG. 1.

As a rule, when a starfish is placed upon its dorsal side, the arm whose tube feet first touch bottom determines the course of the righting. This arm begins at its distal end to twist the

[1]Loeb, "Comparative Physiology of the Brain," Chapter 3.

dorsal side upward, and as rapidly as the twisting is accomplished the tube feet secure a hold on the bottom. Next, if not simultaneously, the arm adjacent to the ventral side of the arm which is obtaining a hold, twists, so that the ventral surfaces of the two arms face each other, and secures itself in the same manner as the first. If *A* and *B* have in this way attached themselves to the bottom, inhibiting impulses are sent to *C* and *D*. The latter release themselves if they have already seized the bottom, rise ventrally, dragging *E* which either remains passive or bends dorsally, even catching the bottom with its tube feet in some cases. The righting is completed by *C*, *D* and *E* passing over *A* and *B* and attaching.

This simple and useful method of righting may be modified by (1) inequalities in the length of the arms, (2) injuries to certain of the arms, (3) any initial twist an arm may have due to its position before the animal was laid upon its dorsal side.

As to (1) short arms are more sluggish than ones of normal length, (2) injury to an arm inhibits the active twisting and seizing of the surface with the tube feet of this arm, (3) if an arm is partially twisted its tube feet reach the bottom more quickly than they otherwise would. As a result we have the following modifications of the normal method of righting.

1. If four arms are injured, their activity is inhibited and the righting is accomplished by the one uninjured arm. It may force an adjacent arm to coördinate weakly.

2. If *A* and *C* (Fig. 1) twist so as to face each other with their ventral surfaces, *B* receives two impulses, from opposite directions, to coördinate, and therefore does not twist either way but bends under dorsally, allowing *A* and *C* to accomplish what *A* and *B* did in the normal case. The same result may be brought about by injuring *B*, *D* and *E*.

3. Sometimes inhibitions are weak and *A*, *B*, *C* and *D* may all remain attached, *C* and *B* facing ventrally toward *A* and *D*. *E* alone is inhibited and the righting is accomplished by *A* and *B* walking backward under *C* and *D*.

I found, as Jennings noted, that in a few cases a starfish persistently refused to use a certain arm for initiating the righting movements. In most cases this was clearly due to an injury or

malformation of the inactive arm. According to the author cited, such a starfish could be "taught" to use the idle arm by giving the animal a large number of "lessons" (180 in one case) in which arms ordinarily active were prevented from taking hold by "stimulating their tube feet with a glass rod" whenever they attempted to attach themselves.

I was able to compel starfish of this sort to use the idle arm by injuring the active ones in the following ways: (1) Irritating the ventral groove of the arm by rubbing it with a glass rod, (2) treating the tips of the arm with a few drops of $n/10$ acid. Two or three applications a few minutes apart usually sufficed to render the arm inactive. In this way I was able to "teach" the starfish in one "lesson," spontaneously to use an arm previously inactive. The length of time the lesson was "remembered" depended upon the degree of the injury. It seems evident from this that Jennings' "lessons" consisted merely in inhibitions due to the injury caused by his irritating the tube feet of the active arms. But an inhibition caused by a single or persistent stimulation is not identical with the phenomena of memory manifested in the process of teaching.

Summary.

1. The righting movements of a starfish which has been placed on its dorsal side are due only to the positive stereotropism of the tube feet.

2. An injury to an arm inhibits its being used for the initiation of righting movements.

3. A starfish cannot be taught to use an arm which is ordinarily passive, but by injuring the other four arms these can be prevented from initiating righting movements and the fifth arm then initiates these movements.

I wish to express my sincere thanks to Professor Loeb for his helpful suggestions and criticism.

A SIMPLE COOLER FOR USE WITH THE MICROTOME.

CASWELL GRAVE AND OTTO C. GLASER.

The microtomist's ability to prepare thin paraffin sections, depends, among other things, on the hardness of his imbedding medium, and this, in turn, on the temperature of the laboratory. Usually this circumstance offers no insurmountable difficulties, but there are times and places when this is not true. To meet such conditions several devices have been suggested and used by various investigators, but we know of none so simple, or as little likely to make difficulties, as the one about to be described.

The apparatus, which is shown, set up for action, in Fig. 1, is essentially a hollow truncated pyramid, open at both ends, and suspended in an inverted position from a standard, so adjusted that the lower end of the shoot is at a convenient distance above the knife. At the upper end of the inverted pyramid, and surrounded by it, is a tray whose dimensions are less than those of the base of the shoot. This tray is filled with crushed ice, and from one corner of it a drain leads the water to the escape from the lower end of the air-channel. At that point a rubber tube connects the pipe with a suitable receptacle.

The cooler is easily set up, interferes in nowise with the operator, and is thoroughly effective. When the air of the room strikes the melting ice in the tray, it is chilled and immediately falls between the tray and the walls of the pyramid. In this way a constant stream of cold air pours from the lower end of the shoot, and as this may be placed directly above the paraffin-block and knife-edge, both of these are cooled, and make it possible to cut sections very much thinner than the unmodified temperature of the room would allow.

The extent to which it is desirable to cool the paraffin and knife varies with each specific case, but the cooler is adjustable in at least two ways. In the first place the distance of the block from the end of the shoot can be changed within comparatively wide limits; in the second place the temperature of the air de-

livered may be further lowered by the addition of NaCl to the ice. Other salts can be used should a greater depression of the temperature be necessary.

The following table is the record of a test made at a room temperature of 87.8°F. The material in this particular case could

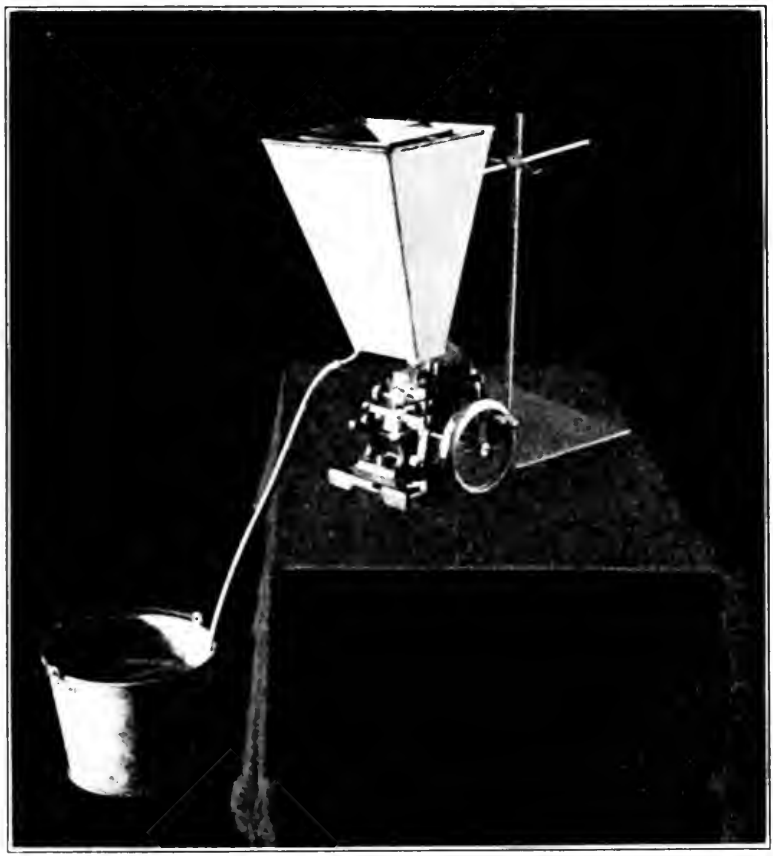

FIG. 1. Photograph of Cooler, by Miss Francis J. Dunbar. Measurements of pyramid: base, 12.5 in. x 8.7 in.; truncated apex, 6.1 in. x 2.1 in. Measurements of ice-tray: 8.8 in. x 3.3 in. Cost, $3.50 without the standard.

not be imbedded in paraffin with high melting-point and satisfactory sections, even as thick as 12 micra, could not be cut. With the aid of the cooler however, a perfect series, 3 micra in thickness, was easily prepared from the same block of 45–48° paraffin.

TEST OF COOLER.

Room temperature ..... 31°C.... 87.8° F

Contents of Tray.	Distance below Mouth of Shoot.		
Crushed ice	6 cm.	24.5	76.1
Crushed ice + NaCl	6 "	23	73.4
Crushed ice	3 "	18	64.4
Crushed ice + NaCl	3 "	17	62.6

Several coolers, varying somewhat in size, but all modeled after our original one at Johns Hopkins University, are now in use in different laboratories. The measurements given in connection with Fig. 1, are those of the cooler at the University of Michigan. This particular one does not have the advantage of a removable ice-pan. In general, size is of little consequence unless it involves too great a reduction in the capacity of the ice-tray, or is conducive to too much absorption of heat by the sides of the pyramid. This latter difficulty is easily overcome by lining the shoot with asbestos paper.

JOHNS HOPKINS UNIVERSITY,
UNIVERSITY OF MICHIGAN.
July 15, 1910.

THE QUESTION OF REVERSAL OF ASYMMETRY IN THE REGENERATING CHELÆ OF CRUSTACEA.

CHARLES R. STOCKARD.

During the summer of 1909 while at the Tortugas Laboratory of the Carnegie Institution I undertook a further analysis of the reversal phenomenon in regenerating specimens of the genera *Alpheus* and *Synalpheus*. These small crustacea commonly called snapping shrimp, on account of their habit of snapping the large chelæ with such force as to produce a surprisingly loud noise, are abundantly found in the "logger-head" sponge and in the holes of disintegrating coral rock on the Tortugas reefs. There are a number of species five of which, *Alpheus formosus* and *armillatus* and *Synalpheus minus* and two other unidentified species of *Synalpheus*, were employed in these experiments.

The several species differ in size and body color but are essentially similar in general structure. The first pair of appendages is decidedly asymmetrical in both sexes. One member of the pair, either the right or the left, is extremely large, in some cases being more than half the size of the body itself. The general type of this chela in the five species follows more or less closely the description given by Wilson,[1] for the great chela of *Alpheus heterochelis*. It is greatly rounded or swollen with transverse grooves on either side of the propodus, varying in depth with the species, and presents characteristic color patterns being tipped with a lively rose color in *Synalpheus minus* while in the other species it is bluish, dark or brown. On the concave side of the dactylus is a swollen knob forming the "hammer" which fits into a corresponding socket on the outer side of the propodus claw. By extending the dactylus and then suddenly snapping the claws together the "hammer" is forced into the socket with the surprisingly loud sound.

Wilson's description further applies in that the large chela has essentially the same structure in both sexes, while the small

[1]Wilson, E. B., "Notes on the Reversal of Asymmetry in the Regeneration of the Chelæ in *Alpheus heterochelis*." BIOL. BULL., IV., pp. 197–210, 1903.

chela shows characteristic sexual differences, we shall be mainly concerned, however, with the fact that the small chela is always typically different from the large chela in shape as well as in size.

Przibram[1] discovered that in several species of *Alpheus* after the removal of the large chela a chela of the small type regenerated from its base while the small chela of the opposite side metamorphosed or developed into a great chela of typical form at the following moult. In other words, the asymmetry was reversed. Further, when both first chelæ are removed they regenerate in their original conditions, no reversal following.

Zeleny[2] found an exactly similar phenomenon to occur after removal of the functional operculum in the worm, *Hydroides*. In this case the rudimentary operculum of the opposite side develops into a functional operculum while a rudimentary organ regenerated from the base of the former functional one. The principle involved in this reversal phenomenon is doubtless the same as that in the crustacea.

Przibram[3] later found a similar reversal to occur in other species of crustacea, while in others the removal of either chela is followed by the regeneration of one of the simpler or smaller type without a regulatory change taking place in the uninjured chela of the other side. In still other cases, as for example the lobster, *Homorus*, a chela similar to the one removed invariably regenerates whether the original chela was a large crusher claw or the slender nipping claw.

The crustacea thus present a series from those forms which regenerate appendages of the type of the ones removed, others which regenerate appendages of the simpler type without a compensatory change taking place in the uninjured chela, and finally such forms as *Alpheus* in which the simpler type of chela is regenerated after the removal of the more specialized chela while the uninjured small chela develops into the more modified type,

[1]Przibram, H., "Experimentelle Studien uber Regeneration," *Arch. fur Entw.-Mech.*, XI., 1901.

[2]Zeleny, C., "A Case of Compensatory Regeneration in the Regeneration of *Hydroides dianthus*," *Arch. fur. Entw.-Mech.*, XIII., 4, 1902.

[3]Przibram, H., "Experimentelle Studien über Regeneration, II.," *Arch. für Entw.-Mech.*, XIII., 1901–1902; "Equilibrium of Animal Form," *Jour. Exp. Zoöl.*, V., p. 259, 1907–1908.

and thus by a sort of compensatory regulation the animal's asymmetrical condition is quickly reëstablished.

Wilson repeated Przibram's experiments on *Alpheus heterochelis* with similar results, but carried the experiments further, hoping to analyze the factors concerned in the reversal process. After removing the great chela the nerve trunk leading to the small chela of the opposite side was clipped in order to test whether there was a nervous control determining the growth of the small chela into a large one. After such an operation the small chela was generally thrown off and only two specimens are said to be beyond question, yet "one of these did not moult quite normally and the other not at all." The evidence, then, does not warrant conclusions as to the cause of reversal of asymmetry in the chelæ. Wilson finally believes that the initial factor that sets in motion the complex process of differentiation of which either side is capable, is primarily only a difference in the amount of material on the two sides. "Removal of the large chela obviously reversed the asymmetry in respect to the amount of material and must, temporarily, at least, lead to a functional nervous difference." Such a suggestion may easily be submitted to experimental test, for example, after removal of the large chela from one side of the body if several posterior appendages be removed from the other side the greater amount of material may still remain on the original large chela side. Fig. 1, *A*, illustrates the operation. Under these conditions will a large chela regenerate from the stump of the old one, instead of arising by a growth of the small first chela of the opposite side?

Again, the proposition may be tested by removing both the great and small chelæ of the first pair and in addition amputating several legs on the side of the large chela; the operation is illustrated by Fig. 1, *E*. The greater amount of material is now on the original small chela side; will this extra amount cause a great chela to regenerate from the small stump instead of from the stump of the great chela which is on the side with less material?

Lastly, when only a portion of the great chela is amputated does it regenerate in the original condition or become a small chela, while a large chela appears on the opposite side through a metamorphosis of the small first chela?

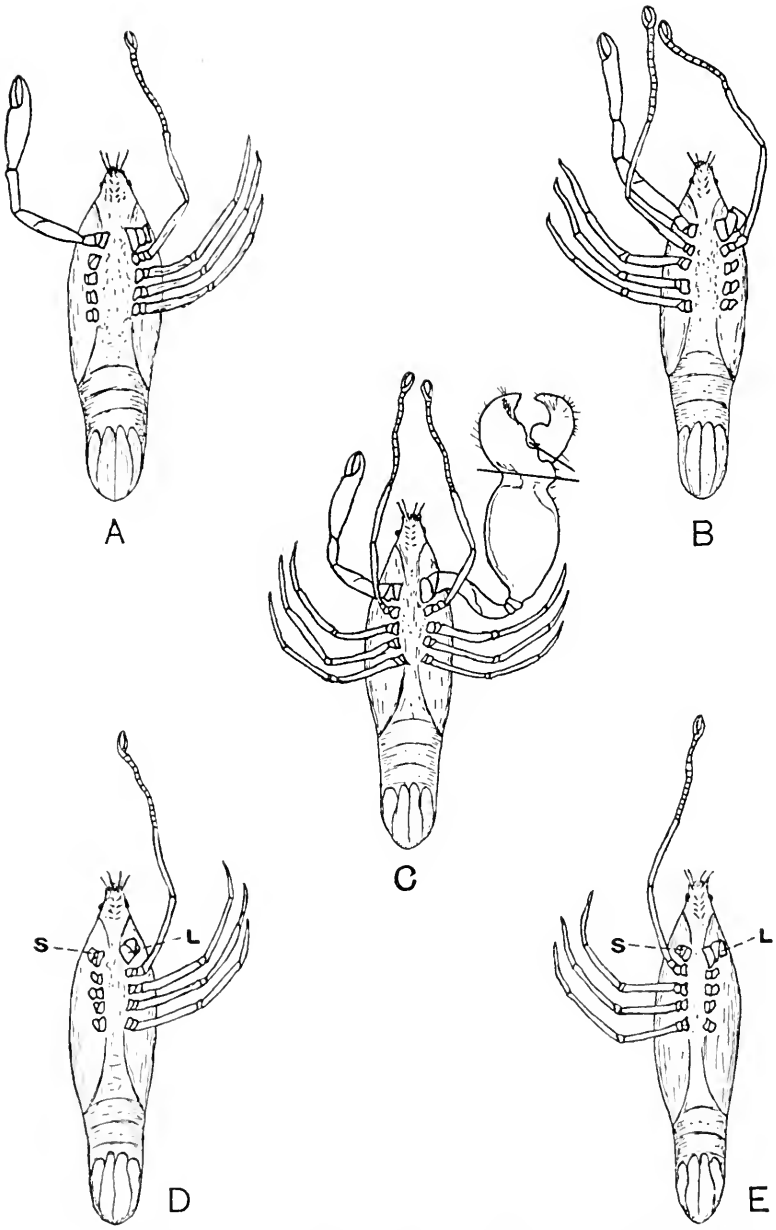

FIG. 1. Diagrams illustrating the manner of operation in the several experiments. *A*, removal of the great chela and the posterior appendages of the opposite side, causing the larger amount of material to still remain on the great chela side; *B*, the opposite operation as a control; *C*, the heavy lines show the places at which portions of the great chela may be cut away without reversal; *D* and *E*, removal of both first chelae and posterior appendages from either the small, *D*, or great, *E*, chela side, to determine the influence of the lateral amount of material on the regeneration of the first chelæ; *L*, large chela stump; *S*, small chela stump.

Aiming towards an answer for these questions a number of experiments were performed the results of which may now be considered.

Fifty healthy specimens representing the five different species were selected, and tested as to their tendency to reverse the asymmetry of the first pair of chelæ during the regeneration following the removal of the great chela. Without exception all of the specimens responded as Przibram had found, a small chela regenerated from the stump of the original great one and the small chela of the opposite side metamorphosed into a great chela. Forty of the specimens favorably survived the experiments.

First Series.

Nineteen individuals had the great chela removed and in addition a number of posterior appendages were amputated from the opposite or small chela side, so as to allow the greater mass of material to remain on the original great chela side. The operation is illustrated by Fig. 1, *A*, and the opposite or control operation by Fig. 1, *B*, or specimens 10, 12 and 31 in the table. Referring to the table the results of such an experiment may be ascertained.

The left side of the table gives the date of the operation, the number of the specimen, and the appendages removed are indicated by *x*, *G* and *S* in the first appendage column indicate the great and small chela. The right side of the table gives the time of moulting and the manner of regeneration, the *r* signifies a new or regenerated leg, *G* and *S* in the first appendage column again indicate the great and small chela. The horizontal lines of the table are so arranged that the appendages on the right side of an individual are given immediately above those on the left of the same animal, *e. g.*, specimen 1 had the great chela and the second leg removed from the right side and the fourth leg from the left side in the first instance. Each specimen, as the table shows, was operated upon a second time during the experiments.

Of the nineteen cases having the great chela removed from one side and other appendages than the first chela from the opposite side the small chela, even though it was on the side of less mate-

rial, retained the power to grow into a large chela of typical form in seventeen cases. One of these cases, specimen 23, is remarkable, since at the first operation the small chela which was on the left side and the other four left appendages were all removed and regenerated at the next moult. After this moult the large chela of the right side was amputated along with the four posterior legs of the left side, thus leaving only the regenerated small chela on the *left side* while the four posterior legs remained on the right side. Nevertheless, the left small chela grew into a great chela and the four more posterior left appendages regenerated for the second time. The case seems an extreme test of the power of one side to regenerate all of its posterior legs for a second time and in addition to change the first chela from the small to the large size and type. Specimen 25 further indicates this remarkable power of the side of the individual with less material to replace all lost parts and at the same time increase the size and type of its first chela.

The remaining two of the nineteen cases, specimens 9 and 18, present the first chelæ equal in size after the moult following the second operation. Specimen 9 had in the first case the great chela removed from the right side and the third, fourth and fifth appendages from the left side. The greater amount of material was, therefore, still on the right side, yet the small first chela of the left side became larger after the moult. The second operation removed the great left chela and the second, third, fourth and fifth legs of the right side. After the moult all of the amputated legs were regenerated but the regenerated left first was small, and the right first appendage had not increased in size. Thus the first pair were symmetrical in respect to size yet the right first or old chela had slightly approached the large chela type. The specimen 18 responded in a closely similar fashion.

The type of the chela is equally, if not more, important than the size since Przibram found the chelae to be of almost equal size in some cases but of reversed type, and the great type invariably increases in size at the following moult.

The experimental evidence in the first case, then, does not support the idea that the side with most appendage material

has most power to produce a great chela of typical size and form. The tendency is to produce a chela of the great size and type from the uninjured first chela, even though this be the original small chela and is located on the body side which has suffered the loss of all other walking appendages. There seems to be no evidence from these experiments to suggest a bilateral distribution of growth energy accompanying distribution of appendage material.

SECOND SERIES.

The question of a bilateral distribution of growth energy related to, or accompanying, the amount of appendage material on a given side was further tested in the following manner. Fifteen specimens were operated upon so as to remove both chelæ of the first pair. It was known that when only these two chelæ were removed that they regenerated in their original condition, a large chela from the base of the original large chela and a small chela from the opposite side. If now in addition to the removal of the first pair of appendages a number of more posterior appendages also be removed from the large chela side, this side will have less appendage material remaining and is, therefore, called upon to regenerate a greater amount of material to replace the posterior legs. Will this side of the body at the same time be more capable of producing a first chela of larger size and specialized type than the opposite side which is called upon to replace only the first chela? Such an operation is illustrated by Fig. 1, *E*, and Fig. 1, *D*, forms a control experiment in which the additional appendages are removed from the small chela side.

The usual idea of regulation would require the side with only the first leg removed to regenerate a large chela while the other side replaced the several posterior legs and produced a small first chela. It is found on examining the table that after an operation to remove both first chelæ and one or more posterior legs on *either* the great or small chela side that six of fifteen specimens regenerated the chelæ of the first pair equal in size, not one individual reversed the type of the first chelæ, and eight regenerated the first chelæ in their original condition as though no additional appendages had been removed. One specimen died before the experiment was completed.

Considering the six specimens that regenerated the chelæ of the first pair equal in size it is important to find that four of these cases, specimens 16, 20, 21 and 39, had the larger number of posterior appendages removed from the side of the original small chela and not from that of the large chela, so that the greater amount of material remained on the large chela side. Such operations were intended as a control for the results following the removal of posterior appendages from the large chela side. Although in these four specimens there was more material on the original large chela side and this side was called upon to regenerate fewer appendages it failed to produce a great chela from the stump of the original one.

In specimen 16 the first chelæ remained equal in size and were both of the small chela type after a second moult. The left chela was then removed and after the next moult the right developed into a great chela and the left again regenerated small. Both first chelæ in specimens 20 and 21 were also of the small chela type, while in specimen 39 the small chela failed to regenerate at the first moult after the operation though a chela of the small type regenerated from the base of the great chela and remained small while the right small chela regenerated at the next moult.

Specimens 31 and 32 had both first chelæ and a number of appendages, four and three, on the great chela side removed. After the moult following the operation the first chelæ were equal in size. Yet again specimens 27, 35 and 38 were operated upon in an identical fashion and after the moult they were able to produce a great chela from the original great chela stump even though this side was called upon to regenerate three other appendages.

Of the fifteen cases tried, therefore, eight regenerate their first chelæ in the original condition of asymmetry while six regenerate the chelæ of the first pair equal in size and usually similar in type whether additional appendages are amputated from the great chela side or from the small chela side. Such a fact would seem to indicate that the amount of appendage material present on either side is an unimportant factor in determining the type of the first chela on a given side, and it seems to show further that there is no clearly evident bilateral distribution of growth energy in these regenerating specimens.

TABLE I.

THE EFFECT OF REGENERATION ON THE ASYMMETRICAL CONDITION OF THE FIRST PAIR OF CHELÆ IN FIVE SPECIES OF *Synalpheus* AND *Alpheus*.*

Date of Operation.	Specimen Number.		Appendages. 1	2	3	4	5	Date of Moult.	Appendages Regenerated 1	2	3	4	5	Remarks.
May 24	1	R	Gx	x			x	June 4	St	r				
		L							G			r		
June 6	1	R		x	x	x		June 16	G		r	r	r	The small chela increases in size and changes its form to type of great chela.
		L	Gx						St					
May 24	2	R						June 1	G					
		L	Sx	x		x			St	r		r		
June 6	2	R	Gx	x				June 15	St	r				
		L		x	x		x		Gr	r	r		r	The third and fifth are only short buds, not fully regenerated.
May 24	3	R			x	x		May 30	G		r	r		
		L	Gx						St					
June 6	3	R	Gx	–				June 15	Gr	–				Only the dactyl removed and regenerated.
		L							S					
May 24	4	R	Gx					May 29	St					
		L							G					
June 6	4	R			x	x	x	June 13	G		r	r	r	Also moulted one day after the operation but had not regenerated
		L	Gx						St					
May 24	5	R						June 4	G					
		L	Gx						St					
June 6	5	R	Gx	x				June 13	St	r				
		L		x	x	x	x		Gr	r	o	o	r	Failure of third and fourth legs to regenerate might account for first chela becoming large.
May 24	6	R	Sx	x	x	x		June 1	St	r	r	r		
		L	Gx						Gr					
June 6	6	R		x	x	x	x	June 12	G	r	r	r	r	
		L	Gx						St					
May 24	7	R						June 3	G					
		L	Gx		x				St		r			Moulted few hours after operation on May 24 and right first chela began to increase in size.
June 6	7	R	Gx					June 13	St					
		L			x	x	x		G		r	r	r	
May 24	8	R	Gx					May 30	St					
		L							G					
June 7	8	R				x	x	June 14	G			r	r	Also moulted two days after operation but no regeneration.
		L	Gx						St					
May 24	9	R	Gx					May 29	St					Moult followed so soon after operation that left first chela had not attained full size, but did so after a few days.
		L			x	x	x		G		r	r	r	
June 7	9	R		x	x	x	x	June 14	S	r	r	r	r	Also moulted 2 days after operation. Right first somewhat similar to great chela type but small.
		L	Gx						Sr					
May 24	10	R						May 30	G					
		L	Gx	x		x	x		Sr	r		r	r	

TABLE I.—*Continued.*

Date of Operation	Specimen Number.		Appendages. 1	2	3	4	5	Date of Moult.	Appendages Regenerated. 1	2	3	4	5	Remarks.
June 7	10	R	Gx	x				June 14	Sr	r				Operated immediately after a moult on June 7.
		L				x	x		G			r	r	
May 24	11	R			x	x		May 30	G		r	r		
		L	Gx						Sr					
June 7	11	R	Gx	x				June 13	Sr	r				
		L			x	x	x		G		r	r	r	
May 24	12	R	Gx					June 2	Sr					
		L		x	x	x			G	r	r	r		
June 7	12	R						June 15	G					
		L	Gx		x	x	x		Sr		r	r	r	
May 24	13	R	Gx				x	May 29	Sr				r	
		L					x		G				r	
June 7	13	R												Died after operation.
		L	Gx											
May 24	14	R	Gx				x	June 1	Gr				o	Only the dactylus and index cut from great chela, no reversal.
		L												
June 7	14	R	Gx	–				June 14	Gr					Second operation and result the same as first.
		L												
May 24	15	R	Gx					June 3	Gr					
		L	Sx						Sr					
June 7	15	R	Gx	x				June 15	Sr	r				
		L			x		x		G		r		r	
May 24	16	R	Gx				x	May 31	Sr				r	After a second moult on June 7 the first chelae were still of equal size.
		L	Sx			x	x		Sr			r	r	
June 7	16	R						June 13	G					The left first chela was removed and right became large and left regenerated small.
		L	Ex						Sr					
May 25	18	R			x			June 1	G		r			
		L	Gx						Sr					
June 7	18	R	Gx					June 14	Sr					Pincer of left first seems slightly nearer the great chela type.
		L			x	x	x		S		r	r	r	
May 25	19	R	Gx					June 3	Sr					
		L							G					
June 8	19	R	Sx		x			June 15	Sr		r			
		L	Gx			x	x		Gr			r	r	
May 25	20	R			x	x	x	June 6	G		r	r	r	
		L	Gx						Sr					
June 8	20	R	Gx					June 19	Sr					Both first chelae of the small type and equal in size.
		L	Sx			x	x		Sr			r	r	
May 25	21	R		x		x	x	May 30	G	r		r	r	
		L	Gx						Sr					
June 8	21	R	Gx	x				June 13	Sr	r				Both first chelae of equal size and small type.
		L	Sx	x		x			Sr	r		r		
May 25	23	R		x		x		June 4	G	r		r		
		L	Sx	x	x	x	x		Sr	r	r	r	r	
June 7	23	R	Gx					June 15	Sr					Second regeneration of all posterior left legs, yet left first became large chela.
		L		x	x	x	x		G	r	r	r	r	
May 25	25	R			x	x	x	June 1	G		r	r	r	
		L	Gx						Sr					

TABLE I.—*Continued.*

Date of Operation.	Specimen Number.		Appendages. 1	2	3	4	5	Date of Moult.	Appendages Regenerated. 1	2	3	4	5	Remarks.
June 10	25	R	Gx		x	x	x	June 17	Gr		r	r	r	Again second regeneration on
		L	Sx			x			Sr			r		one side yet first chela of this side becomes large.
May 25	26	R						June 6	G					
		L	Gx		x				Sr		r			
June 10	26	R	Gx											Did not moult the second
		L				x	x							time.
May 25	27	R		x	x	x		June 4	G	r	r	r		
		L	Gx			x			Sr			r		
June 10	27	R	Gx		x	x	x	June 17	Gr		r	r	r	
		L	Sx						Sr					
May 25	28	R	Gx				x	June 2	Sr				r	
		L							G					
June 8	28	R		x	x	x	x	June 13	G	r	r	r	r	Second pair of legs regen-
		L	Gx	x					Sr	r				erated shorter than usual.
May 25	29	R	Gx					June 6	Sr					Moulted day before the oper-
		L							G					ation.
June 8	29	R			x	x	x	June 16	G		r	r	r	
		L	Gx						Sr					
May 25	30	R					x	May 29	Gr				r	Moult followed so soon after
		L	Gx	x					o	r				operation that little in-
														crease in right chela, no re-
														generation of left, after
														second moult June 7 con-
														dition complete
June 10	30	R	Gx		x	x	x							Died June 16 without moult-
		L	Sx											ing.
May 25	31	R						June 3	G					
		L	Gx	x	x		x		Sr	r	r		r	
June 10	31	R	Gx	x	x	x	x	June 18	Sr	r	r	r	r	First chelæ equal in size and
		L	Sx						Sr					of small type.
May 25	32	R			x	x	x	May 31	G		r	r	r	
		L	Gx						Sr					
June 10	32	R	Gx		x	x	x	June 15	Sr		r	r	r	Both first chelæ smaller than
		L	Sx						Sr					normal but of equal size.
May 25	33	R		x	x	x		May 30	G	r	r	r		
		L	Gx						Sr					
June 10	33	R	Gx	x	x	x		June 16	Gr	r	o	o		Failure of 3 and 4 to regener-
		L	Sx						Sr					rate may account for first
														chela's growth.
May 25	35	R	Gx					May 29	Sr					
		L			x				G		r			
June 8	35	R	Sx					June 14	Sr					Left first chela great though
		L	Gx	x	x		x		Gr	r	r		r	regenerating three other
														legs on same side.
May 25	36	R	Gx					June 3	Sr					Also moulted 2 days after
		L			x	x			G		r	r		operation.
June 8	36	R			x	x		June 15	G		r	r		
		L	Gx						Sr					
May 25	37	R						May 30	G					Moulted again June 6.
		L	Gx			x			Sr			r		
June 8	37	R	Gx		x	x	x	June 15	Gr		r	r	r	
		L	Sx						Sr					

TABLE I.—*Continued.*

Date of Operation.	Specimen Number.		Appendages. 1	2	3	4	5	Date of Moult.	Appendages Regenerated. 1	2	3	4	5	Remarks.
May 25	38	R						May 30	G					
		L	Gx		x	x			Sr		r	r		
June 8	38	R	Gx	x	x	x		June 15	Gr	r	r	r		Also moulted June 9 day after operation.
		L	Sx						Sr					
May 25	39	R	Sx	x	x			June 6	o	r	r			
		L	Gx			x			Sr			r		
	39	R						June 14	Sr	r	r			First chelæ finally regenerated equal in size and remained so after next moult
		L							Sr			r		
May 25	40	R	Sx					May 30	Sr					Moulted again June 6.
		L	Gx			x	x		Gr			r	r	
June 8	40	R	Sx			x	x	June 15	Sr			r	r	
		L	Gx						Gr					

Each specimen was operated upon twice as indicated. R and L following the specimen number signifies right and left sides of the animal; G indicates the great and S the small first chela; x indicates the appendages removed and r the appendages regenerated.

Third Series.

Finally, an attempt was made to determine how large a portion of the great first chela might be removed without causing it to regenerate small; or to cause the small chela of the opposite side to grow into the great type. When a large portion of the chela was quickly clipped off with sharp scissors or a knife the remaining portion was soon thrown off at the breaking joint. The only successful operations consisted in the removal of the dactylus or most distal segment which forms part of the claw, and in the removal of the entire pincer or dactylus and distal end of the propodus, as is indicated by the lines drawn across the chela in Fig. 1, *C*. In the last case a stump-like appendage without a pincer remains.

Following either of these operations the great chela was fully reformed or renewed at the next moult, no reversal taking place.

A small portion of the great chela may then be regenerated in its original form. When the entire chela is removed the small chela of the opposite side invariably grows into a great chela and a small chela regenerates from the stump of the original great one. This reversal of asymmetry may be shifted back and forth for a number of times and occurs in a manner as decidedly pronounced after several operations as it does after the first.

Conclusions.

The power to reverse the asymmetry of the first chelæ when regenerating a great claw in *Alpheus* does not seem to be closely associated with a difference in the amount of material on the two sides of the body nor with a bilateral distribution of growth or regenerative energy.

Although in certain cases there seems to be a tendency to regenerate the chelæ of the first pair equal in size and similar in type, such a tendency is manifest under conditions so varied in respect to the bilateral distribution of appendage material and call upon the powers of regenerative energy that the present conclusion is warranted. The amount of material on a given side of the animal, or the amount of regeneration required of this side are negative factors in determining the ability of the side to produce a great chela instead of a small one.

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BIOLOGICAL BULLETIN

EXPERIMENTS ON COLOR-VISION OF THE HONEY BEE.

C. H. TURNER.

Introduction.

Whether insects can or cannot distinguish colors is a matter of much theoretical importance, for the correct interpretation of the relation of insects to flowers depends upon this answer. Most students of natural selection believed, at one time, that the forms and colors of flowers were adaptations to insect visitors. Lately there has been a reaction based on the general consensus of opinion, among morphological entomologists, concerning the poorness of insect vision. Kellogg[1] writes: "The fixed short focal distance, the incompleteness and lack of detail incident to a mosaic image, and the lack of accommodation (only partly provided for by the shifting of the peripheral pigment) to varying light intensity, which are admitted conditions of insect vision, make it seem difficult to account for the intricacy in pattern common to many flowers on a basis of adaptation to animal visitors of such poor seeing capacity as insects.

"Experimental evidence touching this criticism is singularly meager when one considers the importance of the subject. If insects can accurately distinguish colors, and at some distance, and can perceive the fine details of color-pattern at a very short distance, then the explanation of floral structure and pattern as adaptation to insect visitors has solid foundation for even the amazingly large and varied results which it attempts to explain; if not, it is hard to understand how the explanation is valid (at

[1]Kellogg, V. L., "American Insects," Henry Holt & Co., second edition, revised, 1908, pp. 580.

least in any such all-sufficient degree as commonly held), despite its logical character (in light of our knowledge of the nearly limitless capacity for modification of natural selection) and the abundant confirmatory evidence.

"Most of the experimental evidence so far offered is that included in Darwin's account ('On the Fertilization of Flowers by Insects'); in Lubbock's account of his experiments on honey-bees, familiar because of its presentation in his readable book, 'Ants, Bees and Wasps'; and in Plateau's account of his more recent but less familiarly known experiments with various insects including bees. Both Lubbock and Plateau are investigators ingenious in device, keen in deduction, and of unquestioned scientific honesty. Yet their conclusions are a direct contradiction. Lubbock believes that bees recognize colors at a considerable distance, that they 'prefer one color to another, and that blue is distinctly their favorite.' Plateau finds that neither the form nor the brilliant colors of flowers seem to have any important attractive rôle, 'as insects visit flowers whose colors and forms are masked by green leaves, as well as to continue to visit flowers which have been almost totally denuded of colored parts'; that insects show no preference or antipathy for different colors which flowers of different varieties of the same or of allied species may show; that flowers concealed by foliage are readily discovered and visited; that insects ordinarily pay no attention to flowers artificially made of colored paper or of cloth whether these artifacts are provided or not with honey, while, on the contrary, flowers artificially made of living green leaves and provided with honey are visited (from the attraction of the 'natural vegetable odor'). From these observations Plateau concludes that 'insects are guided with certainty to flowers with pollen or nectar *by a sense other than that of vision and which can only be that of smell*,' and finds particular proof of this in the facts, according to his observations, (1) that insects tend, without hesitation, towards flowers usually neglected by reason of the absence or poverty of nectar, from the moment that one supplies these flowers with artificial nectar, represented by honey; (2) that insects cease their visits when one cuts out the nectary without injuring the colored parts, and re-begin their visit if one replaces

the destroyed nectary by honey; (3) that it suffices to attract numerous insects if one puts honey on or in normally anemophilous flowers, simply green or brown in color, which are normally practically invisible and almost never visited by insects; and (4) that the visiting of flowers artificially made of fresh green leaves and containing honey demonstrates plainly the rôle of the sense of smell.

"It must be said that, despite many just criticisms that may be made on the character of his experiments, Plateau has made necessary more experimentation for the relief of the general theory that floral adaptation of color is due to color preferences of insect visitors."

Forel[1] and von Buttel-Reepen[2] are opposed to Plateau's views, but Bethe[3] is in accord with Plateau.

To test his conclusions, Forel repeated, in the following manner, Plateau's dahlia experiment. (1) Paper dahlias were distributed among some dahlias from which a large number of bees were collecting honey. The bees paid no attention to these artifacts. (2) Honey was placed on these artifacts, and, by skillful manipulation, brought to the attention of one of the bees. Immediately that bee neglected the real dahlias for these artificial ones. (3) Gradually all of the bees neglected the dahlias for those artifacts with their inexhaustible supply of honey — inexhaustible because it was constantly replenished by Forel. (4) The artifacts were removed. After a lapse of several days, similar artifacts, but containing no honey, were scattered among those dahlias. Immediately the bees neglected the dahlias for the artifacts, which they searched for honey. Forel thinks this experiment shows that bees have space, form and color perception.

Von Buttel-Reepen bases his opposition to Plateau's views largely upon information furnished him by Herr Roth, leader of the Baden bee-keepers school, and a teacher named Staehelin.

[1]Forel, Aug., "Die psychischen Fachigkeiten der Ameisen und einiger anderer Insekten," Muenchen, 1901. "Ants and Some of their Instincts," *Monist*, vol. 14, 1903–4.

[2]Buttel-Reepen, H. von, "Sind die Bienen Reflex-maschinen?, Experimental Beiträge zur Biologie der Honigbiene," *Biol. Centralbl.*, Bd. 20, 1900. "Are Bees Reflex Machines?" translated by Mary H. Geisler, Medina, O., 1907.

[3]Bethe, A., "Die Heimfähigkeit der Ameisen und Bienen zum Theil nach neuen Versuchen," *Biol. Centrlbl.*, Bd. 22, 1902.

Von Buttel-Reepen states: "We have seen above that the flight [of bees] becomes very unsafe in the dusk; therefore it is evident that gloomy weather influences considerably the ability to orient. 'One of my former neighbors,' Roth says in his communication, 'painted the gable of his house over the apiary with a sky-blue (luftblau) color. The same bees which always flew over the gable, on the next dark day, bumped against it with their heads, trying to fly through it.' A teacher, Staehelin, made the following observations: A weak after-swarm, mostly of young bees from a hive painted blue, dispersed among the masses of humming bees which were just taking their flight of orientation out of the other hives (which, as is usually the case in Germany, Switzerland, and Austria, were standing close together), and settled here and there in clumps. After a short time they flew back to the bee-house; but only a few found the right hive; the rest flew to other colonies, and to which? Only to those where a blue door invited them did they attempt an entrance, but nowhere else. Unfortunately they were so hostilely received that the ground in front of all of the blue hives was covered with bees."

Bethe had a swarm of bees lodged in a brown hive which rested on a table. He painted the outside of the hive blue and covered the table with green branches. Instead of the backgrond of trees, he substituted one of white and yellow flowered cloth. No change was produced in the home-coming of the bees. This Bethe considers conclusive proof that bees are not guided home by memory picture contributed by the eyes.

So far as my knowledge goes, M. Gaston Bonnier[1] is the only recent investigator who furnishes any experimental evidence that supports Bethe's view. He found that bees, the eyes of which had been rendered opaque with pigmented collodion, would pass direct to the hive from any distance less than three kilometers. This observation, which is not in harmony with Forel's experience,[2] supports Bethe's contention, but it has no direct bearing upon color vision.

The purpose of this paper is not to discuss the homing of the

[1]Bonnier, M. Gaston, "Le sens de la direction chez les abeilles," *C. R. Acad. Sci.*, Paris, T. CXLVIII., 1909, pp. 1019–1022.

[2]Forel always found that bees, the eyes of which had been rendered opaque, could not find their way home.

honey bee; but, by means of simple experiments, to throw some light upon the question "Can bees distinguish colors?"

Description of the Experiments.

The following experiments were performed in a large field just west of O'Fallon Park, St. Louis, Mo. The white sweet clover (*Melilotus alba* Lam.), with its long racemes of white papilionaceous flowers, was abundant in dense patches; but there were a few vacant places in the field. Foraging bees were visiting this white melilot in large numbers.

Series I. (July 12, 2 P.M.).

The discs used in this series of experiments were cut from colored cardboard, and each was six centimeters in diameter.

Experiment 1.— *I placed six discs of red cardboard on the top of rods that had been erected in the midst of a patch of white sweet clover. The rods were so adjusted that the top of each was about on a level with the tops of the weeds. Six similar discs were attached, at different heights, to the branches of the weeds. Honey was placed on all of these discs.*

More than an hour passed by and no response was made to these discs by the bees; but both flies and wasps visited them. The weeds were full of bees that were continuously flying to and fro in the immediate vicinity of these artifacts with their copious supply of honey. Were the odor of honey alone sufficient to attract bees reflexly, these bees should have been attracted early. After waiting half an hour, I decided to force the bees to attend to my artifacts.

Experiment 2.— *A bee was captured in a wide-mouth bottle and the bottle, with the cork removed, inverted over one of the red discs of experiment 1, until the bee dropped upon the disc; the bottle was then removed. This was tried with six different bees.*

In each case the bee always ascended to the top of the bottle and attempted to escape. After several futile efforts it would drop, either by accident or from exhaustion, upon the disc. At that moment, I always removed the bottle. Immediately the bee would leave never to return. Some of the bees fell into the honey; but, even in that case, they did not return.

EXPERIMENT 3.—*A branch containing blossoms on which a bee was foraging was gently removed from the plant and so manipulated that the bee was less than two centimeters from the honey of one of the discs of experiment 1. This was tried with six bees.*

In no case did the bee pay any attention either to the honey or to my discs. The bee always left immediately and went to one of the blossoms of the melilotus.

EXPERIMENT 4.—*Whenever a bee alighted on a blossom near one of the discs of experiment 1, I gently moved the sprig until the bee was brought to within less than two centimeters of the honey. This was tried with a dozen bees.*

No response was made to the honey.

In a cluster of weeds about a yard from the one in which most of my discs were located, I had placed, at the beginning of this series of experiments, a red disc so copiously supplied with honey that it overflowed upon the weed. This disc was so situated that by simply raising my eyes I could see it. Although the melilotus was swarming with bees, that disc remained in that place for nearly two hours before receiving its first visit from a bee. At that time, however, a bee hovered at the edge of the disc and began to sip the honey. It then alighted on the edge of the disc and continued to sip the honey. Almost immediately another bee flew up to this one. They both circled about for a moment and then alighted on the disc; one on the edge and the other near the center of the upper surface. From this time on, all of my attention was focused upon this plant.

EXPERIMENT 5.—*Near this disc was a blossom which I had wet with honey. While the two bees mentioned in the above experiment were foraging on disc one, a bee alighted on this blossom. I gently moved the sprig until the bee was within about a centimeter of the two bees just mentioned.*

It left the blossom and, alighting on the disc, began to forage.

EXPERIMENT 6.—*While these bees were imbibing honey, I attached two other red discs, each supplied with honey, to other branches of the weed.* (For descriptive purposes, starting with the disc upon which the bees were feeding, we will designate them disc one, disc two, disc three.)

One by one, the three bees on disc one departed for the hive.

On leaving, each hovered a moment above the disc, circled around it, made two or more short circles about the weed, then, ascending, departed. In about five minutes, two of the bees had returned to the weed. They did not visit any of the blossoms; but, after circling in the vicinity of the disc for a moment, alighted on the disc and began to imbibe the honey. They arrived less than half a minute apart. After securing a supply of honey, they departed in the same manner that they did before. In less than five minutes, the two had returned. One of these, after arriving at disc one, left it and went to disc two and obtained honey. These three bees were watched carefully for half an hour. In that time fourteen visits were made to the red discs; eleven to disc one and three to disc two. No visits were made to disc three. On two occasions three and on two other occasions two bees were on disc one at the same time. On each of these occasions, before departing for the hive, the bees always explored the neighborhood of the disc.

For the half hour or more that I was conducting this experiment, I was too much occupied to pay any attention to the discs on the other weed. I now watched them continuously for fifteen minutes. Although the weed was alive with bees, no bee visited the red discs. Had any of the bees discovered those discs and had they begun to collect honey from them, some of them should have made a return trip while I was watching. It is reasonable to conclude that no bees had visited them.

Thus, although over a dozen discs, well supplied with honey, were exposed, on weeds that were alive with bees, for fully three hours, yet only three bees visited those discs and their visits were confined to two of them![1]

EXPERIMENT 7.—*On leaving for home, all of the discs were removed from the field except the three from which the bees were collecting honey. On those discs I placed all of the honey that they would hold.* This was done hoping that, before dark, other bees, by imitation, would learn to collect honey from those discs.

[1] I have tried this same type of experiments at other times. Although considerable time always elapsed before the discs were attended to by the bees, yet the time was seldom as long as this.

Series II. (July 13, 8 A.M.)

On my arrival at the field this morning, I noticed that all of the honey had been removed from the discs; and, in two cases, that much of the color had been removed in spots. It looked as though the bees had attempted to carry off even the paper that had been saturated with honey.

EXPERIMENT 8.—*Among the branches of the same plants of melilotus from which, yesterday, a few bees learned to collect honey, I placed six red and six blue discs. Two of the red discs (1, 4) and one of the blue were attached to the tops of rods five feet high (the height of the weeds), the others were pinned, at different levels, to the branches of the weed. In the center of each red disc, honey was placed. The red discs were numbered from 1 to 6, the blue from 7 to 12. There was no honey on the blue discs.*

Almost immediately a bee alighted on disc one. These discs were watched continuously for a little less than a half hour. During that time no bees visited the blue discs, but they made thirty-nine visits[1] to the red discs. These visits were distributed as follows: disc one, seven; disc two, two; disc three, nine; disc four, seven; disc five, six; disc six, eight. The bees visited the discs on the rods (1, 4) just as readily as they did those attached to the weeds; they visited those high up (1, 2, 4, 6) just as frequently as they did those low down (3, 5). Whenever a bee was ready to depart for the hive, it always made, in the manner already described, a careful orienting flight. From now on the bees began to visit the red discs in such large numbers that it was impossible to keep an accurate record of the number of visits Sometimes as many as ten bees would visit the same disc at the same time.[2] The significance of this marked change in the behavior of the bees will be discussed later.

EXPERIMENT 9.—*I selected a red disc from which four bees had been collecting honey and, while the bees were away, placed it about six inches lower on the plant. In its place I placed a blue disc. The blue disc did not have any honey on it.*

[1]In all of these experiments, whenever a bee alighted on one of my artifacts, it was counted a visit, whether it was the arrival of a new bee or a return visit of a former visitor.

[2]When these experiments were first planned, it was my intention to mark each bee that participated; but, at this stage of the work, I realized that such a procedure would be impracticable and my paint and brush were put away.

The bees that arrived at the weed in the vicinity of the blue disc dropped at once to the red disc, without even pausing before the blue.

EXPERIMENT 10.—*I placed three more rods in the midst of the same weed; on two of them I placed blue discs without honey, and on the other one a red disc with honey.*

At the end of fifteen minutes, all of the red discs were being visited by numerous bees; none of the blue discs were being visited.

EXPERIMENT 11.—*Two rods were placed so near each other that a space of not more than two centimeters separated the discs. One disc was red and the other blue. The red disc was supplied with honey, the blue was not. After they had been in one position for fifteen minutes, the red disc was placed where the blue had been and the blue placed in the place from which the red had been taken.*

During the few minutes that these discs were under observation, many bees visited the red disc; on one occasion, three bees were obtaining honey from it at the same time. Only one bee visited the blue, and, evidently, she was not foraging for honey. She spent at least ten minutes on the disc and most of that time was spent in one place. A part of the time she was rubbing her legs against the edge of the disc, the remainder she seemed to be simply resting.

EXPERIMENT 12.—*Five centimeters from a red disc containing honey, I placed a blue disc containing honey.*

During the ten minutes that these discs were watched, twelve visits were made to the red disc and only one to the blue. On three different occasions, there were three bees on the red disc at the same time. It was at the close of the ten minutes that the first bee visited the blue disc.

Just as soon as the bee had imbibed the honey and left the blue disc, the disc was replaced with a blue disc that was not supplied with honey.

During the next five minutes, five visits were made to the red and none to the blue. One bee hovered momentarily above the blue and then went to the red.

EXPERIMENT 13.—*In a different place from that where experiment twelve was performed, I arranged, close together on rods, one blue*

and two red discs. On the blue disc and on one of the red discs honey was placed.

During the time these discs were under continuous observation, fifteen visits were made to the red disc that was supplied with honey, one bee alighted on the red disc that did not bear honey, and three bees alighted on the blue disc. These three bees visited the blue disc at the same time; one bee alighted on the disc, and then almost immediately the other two followed.

While the three bees just mentioned were on the blue disc, the rod supporting that disc was gently removed to a portion of the melilotus patch that did not contain any of my experimental discs.

One by one, the bees made a careful orienting flight and then flew away. These discs were no longer kept under continuous observation; but, at regular intervals, they were visited and the honey replenished. On those occasions I would watch each disc for about five minutes. On each trip I found three bees visiting the blue disc. The disc might be free from bees when I arrived; in a short time, however, three bees would arrive. They did not arrive simultaneously; but, before the first arrival had left two more would be there. I therefore concluded that the same three bees that discovered the honey on the blue disc had continued to visit it, and that no other bees had grasped the significance of that blue disc. (Unfortunately, for the reason mentioned above, these bees were not marked and one cannot be absolutely certain of their identity; but, from a knowledge of the habits of bees when foraging and of the time required to make a trip to the hive, I feel certain that they were the same bees.)

EXPERIMENT 14.—*While several bees were collecting honey from one of the red discs that capped one of my rods, the rod was gently carried fifteen feet in the direction of the hive and erected in another patch of melilotus.*

One by one, the bees made a careful orienting flight and then flew away. In a short time they had returned. Often eight or ten bees would be on the disc at the same time. While I was taking my notes, some of the bees hovered within a short distance of the small pad (13 × 8 cm.) on which I was writing, as though they were examining it. From now on this behavior was common.

EXPERIMENT 15.—*While ten bees were foraging on the red disc*

used in experiment fourteen, the rod was gently carried fifteen feet nearer the hive and erected in a place that was free from tall weeds; even the grass had been cropped short by a horse that had been grazing there.

The bees, on leaving, hovered about the disc a long time, even examining the cork to which the disc was pinned. Then, after describing a shallow spiral, they flew away. In a short time six had returned to this disc. On her return, each bee flew to the cork first and then to the top of the disc. I was now forced to leave the experiment for half an hour. On my return, the disc was being visited by numerous bees. During the five minutes that I watched it, twenty-six visits were made to it. There were always from six to ten bees on the disc.

EXPERIMENT 16.— *From the melilotus weed in which the first experiments of this series were performed, I gently removed a rod which was capped with a red disc upon which ten bees were foraging and erected it fifteen feet further away from the hive, in another patch of white sweet clover.*

The bees on leaving made a careful orienting flight. I left the disc for about twenty minutes. On my return, I found seven bees resting on the disc and imbibing honey.

EXPERIMENT 17.—*At that end of the field which was most distant from the hive, and at about fifty yards from the weeds in which the first experiments of this series were performed, there was a large patch of the same plants, from the racemes of which numerous bees were collecting honey. In the midst of these weeds, but well exposed, I placed a red disc upon which I had poured some honey.*

I remained by this rod for twenty minutes, but no bee approached it. At intervals of ten minutes, I made six visits to this disc. Each time I remained five minutes. At no time did I find any bees visiting the disc.

EXPERIMENT 18.—*In a space free from tall weeds, about thirty yards nearer the hive than the weed in which the first experiments of this series were performed, I erected one of my five foot rods and on its top placed a red disc well supplied with honey.*

At intervals of ten minutes, I made four visits to this disc. The first time I found one bee on the disc; the second time, three; the third time, two, and the fourth, four.

At this stage, I removed all of the discs from the field except the one used in this experiment.

At intervals of ten minutes, I made two additional visits to this disc. On the first trip I found ten bees on the disc; on the second trip, I found eight.

On leaving for home all of the discs were removed from the field.

Series III. (July 14, 7:30 A.M.)

Apparatus.—The cornucopias used in this series of experiments were made in the following manner: A piece of cardboard, colored on both sides, was cut the shape and dimensions shown in Fig. 1. It was folded along the dotted lines and the flaps

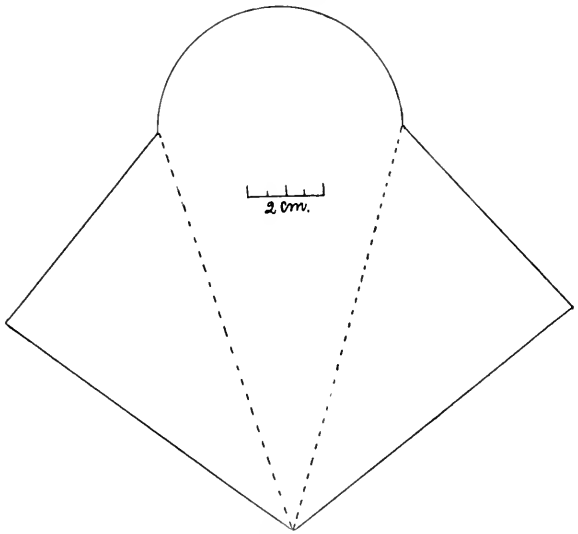

FIG. 1.

fastened where they lapped. About one centimeter of the apex of the cone was bent over and fastened. When finished, each cornucopia was nine centimeters long, with an elliptical lip six centimeters wide and three centimeters high. The lip was used for attaching the cornucopia to some support. Incidentally it furnished a platform for the bees. Some of the cornucopias were red and some were green.

EXPERIMENT 19.—*In the weeds that were the seat of the experiments of yesterday, several cornucopias were arranged at different*

levels. Half of these were red and half were green and they were arranged in pairs, one red and one green constituting a pair. The openings of both members of a pair faced in the same direction. All of these cornucopias were attached to parts of the weed. The red ones were supplied with honey, but the green were not.

Immediately after I had pinned the first red cornucopia to the bush, a bee entered it and began to collect the honey. Two of these pairs were kept under observation for nearly an hour. During that time numerous bees entered the red cornucopias, but not a bee entered the green ones.

EXPERIMENT 20.—*Five red cornucopias, each containing honey, were arranged on rods in the following manner: A, in a patch of melilotus about fifty yards further from the hive than the weeds that were the site of experiment nineteen; B, C, D, arranged in a line extending from the patch that was the site of experiment nineteen towards the hive, B twenty feet from the patch, C thirty-five feet, and D fifty feet; E sixty yards from the same patch mentioned above in a line which, at the experiment patch, made an angle of 45° with the line containing B, C, and D. Each of these cornucopias was visited once every twenty minutes, at which times it was watched closely for three minutes. The number of bees in the cornucopia when I arrived were counted, the number to arrive after I did were noted and the sum of the two numbers recorded.*

The results of the above experiment are recorded in the following table:

	Name of the Cornucopia.				
	A	B	C	D	E
Number of bees observed on trip 1	0	1	7	1	0
Number of bees observed on trip 2	0	8	2	8	0
Number of bees observed on trip 3	0	12	6	6	0
Number of bees observed on trip 4	1[1]	21	13	11	2[1]
Number of bees observed on trip 5	1	X	12	16	1
Number of bees observed on trip 6	0	X	X	X	0
Number of bees observed on trip 7[2]	1[3]	21	X	X	6

[1]Before leaving the bee made a careful orienting flight. She examined the cornucopia on all sides several times, reentered it three times, and, after describing a corkscrew curve, flew away to the hive.

[2]There was an interval of an hour between trip six and trip seven.

[3]Two other bees hovered near, but did not enter. The one that entered left immediately. The cornucopia was swarming with ants.

X means that the bees were so numerous that it was impossible to make an accurate count.

EXPERIMENT 21.—*Side by side, on one of the branches of the melilotus weed upon which most of these experiments were conducted, I arranged a red and a green cornucopia, and placed honey in each.*

During the first five minutes that these were under observation twenty-five bees entered the red cornucopia and three the green.

EXPERIMENT 22. *On rods erected in the open space between the experiment weed and the hive, and about three feet from disc C of experiment 20, a red and a green cornucopia were arranged side by side. Each contained honey.*

During the first five minutes that these cornucopias were watched, sixteen bees entered the red cornucopia and four the green.

EXPERIMENT 23.—*The green cornucopia of experiment 22 was replaced by a red cornucopia which did not contain, and never had contained, honey. This placed two red cornucopias side by side, one containing honey and the other empty.*

During the five minutes that these cornucopias were under observation, so many bees entered the red cornucopia which contained honey that it was impossible to count them; five entered the empty red cornucopia.

The empty red cornucopia was now placed where the one containing honey had been and the one containing honey placed in its stead.

During the first five minutes that they were observed, so many bees entered the cornucopia which contained honey that it was impossible to count them; many bees hovered around the empty cornucopia, but none entered.

The cornucopia that contained the honey was removed, the bees shaken out, and the cornucopia put out of sight. This left an empty red cornucopia in a part of the field which, for more than half an hour, had contained at least one red cornucopia which was well supplied with honey.

At first the bees circled around the cornucopia, presently one entered and then left immediately. Within ten minutes twenty-five bees had entered the cornucopia. (This does not mean twenty-five different bees, for the same bee entered more than once and was counted each time.) At first each bee left as soon as she had reached the inner depths of the cornucopia. Soon, however, the bees began to enter so rapidly and in such large numbers

that it was impossible for those that had reached the inner depths to leave without a struggle; and, in less than thirty minutes, the cornucopia was packed almost full of struggling bees, and numerous others were hovering around the mouth, seeking a place to enter.

EXPERIMENT 24.—*Ever since the beginning of this series of experiments, the red cornucopias on the melilotus had been kept well supplied with honey. At this time the weed contained eight red cornucopias and an equal number of empty green ones. Into the upper portion of this weed, I placed an empty red cornucopia.*

During the five minutes that this cornucopia was watched, twelve bees entered, one at a time, tarried a moment and then left.

EXPERIMENT 25.—*In the open, three feet from the empty red cornucopia of experiment 23, I placed, on a rod, an empty green cornucopia. In this place, earlier in the morning, there had been a red cornucopia well supplied with honey.*

During the ten minutes that this cornucopia was observed, many bees hovered around it; one alighted on the front platform, but none entered.

All of the cornucopias were removed from the field except the empty red cornucopia of experiment 23 and the empty green one of this experiment. This left only two cornucopias in the field; one red and one green, neither of which had ever contained honey.

This green cornucopia was watched continuously for ten minutes. During that time many bees hovered around the green cornucopia; two alighted on the front platform, and one entered. At the close of the ten minutes, I walked over to the empty red cornucopia and found it almost full of struggling bees, and numerous other bees were hovering around the entrance seeking admittance.

Series IV. (July 15, 3 P.M.)

This series of experiments was conducted with special cardboard boxes; each consisting of a rectangular outer case (8 × 5.2 × 2.5 cm.) with a porch-like extension in front and open ends, into which there was shoved, from the rear, a cardboard tray 5.5 × 5 × 2.4 cm. Near one side of the front end of this tray an entrance was made. In most cases this entrance was a rectangular opening 2 × 1.2 cm.; in a special case it was circular and 1.5

cm. in diameter. The tray was shoved in from the rear until its rear end was just inside of the rear edge of the outer case.

In constructing the outer case, a piece of cardboard was cut the shape and dimensions of figure two, folded along the dotted lines and glued where the sides overlap. In constructing the inner tray, a piece of cardboard was cut the shape and dimensions of figure three, folded along the dotted lines and glued, by the flaps, to the inner portions of the adjacent sides.

EXPERIMENT 26.—*In the same weed that has been the site of most of these experiments, I placed one green and two red boxes. The green box was placed near one of the red boxes. The red boxes contained honey, the green was empty.*

It had been raining all morning and it was still quite cloudy, and only a few bees were afield. About five minutes after the beginning of the experiment a bee noticed one of the red boxes. She examined it carefully from all sides, found the entrance

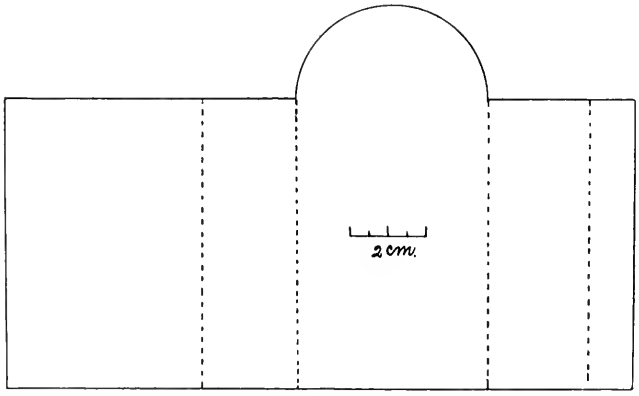

FIG. 2.

and entered. Soon after she entered, a heavy shower of rain began to fall and I took shelter under a tree. On my return (about fifteen minutes later) I found a large digger wasp in box number one and bees visiting red box number two. As long as that wasp remained in box number one, no bee would enter it. During the time that these boxes were under continuous observation, fifteen bees visited red box number one and twenty-eight visited red box number two. Towards the close of this experi-

ment, some of the bees would fly into the tray without first alighting on the portico. Not once was the green box visited by a bee.

EXPERIMENT 27.—*On my return after the rain mentioned in experiment 26, a watch-glass, seven centimeters in diameter, containing honey, was placed on the ground within a foot of the weed in which the boxes mentioned in experiment 26 were located. It was left uncovered; but, on such a cloudy afternoon, it was not conspicuous.*

During the time that I was watching experiment 26, no attention was paid to the watch-glass. At the close of that experiment the watch-glass was observed continuously for ten minutes. During that time not a single bee visited the watch-glass. Since the trip to the hive required less than five minutes, any bee that had succeeded in finding this watch-glass full of honey would have made at least one visit while I was watching; hence it is logical to conclude that no bee had visited it.

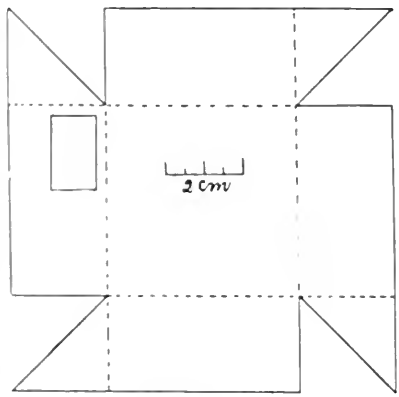

FIG. 3.

At this point rain caused a recess until eight A. M., July 16. On leaving for home, all of the boxes were removed from the weeds and the watch-glass taken from the ground.

EXPERIMENT 28.—*Side by side, in the same weed that has been the seat of the majority of these experiments, I placed two red experiment boxes; one contained honey and the other was empty.*

As soon as I appeared on the scene, the bees began to hover about me, and, before I could pin the red box with its supply of honey to the weed, a bee had entered its tray. In a few minutes so many bees were visiting the red box that contained the honey that it was impossible to count them. Frequently bees would enter the empty red box, but they would not tarry long.

EXPERIMENT 29.—*In the open space between the experiment weed*

and the hive, I arranged, on poles, about ten centimeters apart, three red boxes containing honey, one empty red box and one empty green box. The arrangement of the boxes in the group was altered once in ten minutes.

These boxes were under continuous observation for about an hour. Immediately the bees began to visit the boxes that contained honey in such large numbers that it was impossible to count them. Occasionally a bee would enter the empty red box and frequently they would hover in front of the entrance to its tray. No bee entered the green box, although occasionally a bee would alight on its top and pause long enough to clean its legs on an edge of it, and frequently one would pause a moment before some portion of the box. Whenever the honey was exhausted from one of the trays, the number of visitors would drop off. Seldom would a bee pass through the entrance; frequently one would hover momentarily before the door and then pass on. As soon as I had replenished the honey, the bees would begin to revisit it.

EXPERIMENT 30.—*After the above experiment had been under way for an hour, all of the boxes were removed from the field, except the empty red and the empty green box of experiment 29.*

Immediately the bees began to enter the red box more frequently than they had hitherto; as soon as one got well inside, it would leave. After a lapse of a few minutes, the bees began to rush for the entrance in such large numbers that those that had entered and wanted to leave could not do so without a struggle. As a result the tray and the portico were crowded with struggling bees, and numerous others were hovering about the entrance, seeking admittance.

At first no bees entered the green box, although many circled about it. After a lapse of ten minutes a few began to enter. During the period of observation, ten were noticed to enter it and leave immediately, and about twice that number were noticed to alight in the portico.

EXPERIMENT 31.—*Standing about three feet from the above boxes, I held, in my hand, a red box containing honey.*

Immediately a few bees approached and entered the box. I held the box in my hand for about five minutes. Throughout

that period there were from one to four bees inside of the box all the time.

EXPERIMENT 32.—*Standing in the same place mentioned in experiment 31, I held an empty red box in my hand. This box had never contained honey.*

Several bees approached and hovered around the box, and two entered.

INTERPRETATION OF THE EXPERIMENTS.

Vision or smell or some combination of the two are instrumental in guiding insects to flowers. Lately some observers have suggested that vision plays no part in this behavior; indeed, it is claimed that it is not even olfactory perceptions, but odors acting reflexly that lead insects to flowers. No one who has made observations for himself would think of claiming that smell plays no rôle in insect behavior; but, the experiments described above show conclusively that odors, acting reflexly, do not lead bees to flowers. In localities where bees are not accustomed to obtain honey from anything but flowers, honey may be placed on small discs (Ex. 1, 17), or in open vessels (Ex. 20–*A* and *B*, 27) and left exposed for a long time without being responded to by the bees. Bees, captured in bottles and turned loose upon discs that are well supplied with honey, will usually depart without paying any attention to the honey (Ex. 2). If a flower upon which such a bee is foraging is so manipulated as to bring the bee in close proximity to the honey of one of those discs, she will usually depart without responding to either the honey or the disc (Ex. 3, 4). It is incredible that an odor of honey too weak to attract bees a few millimeters off is able to entice them from several meters in the air. Then, too, each of my discs contained more than a thousand times as much honey as any one of those flowers did nectar, and the cornucopias that I used contained more than the discs; yet the bees passed by the feast of honey prepared for them to sip the meager supply of nectar stored in the neighboring small flowers. Such behavior would be impossible if their movements were controlled by the honey-odor acting reflexly. To claim that the nectar of the flower has a greater attractive power than the honey would be illogical for: 1st, honey is concentrated nectar; and 2nd, these same bees, after they have

learned to collect honey from objects other than flowers, will visit such objects as soon as they are attached to the support (Ex. 8, 19), at times they will even enter them while they are being attached to the support (Ex. 28), or they may even enter such an object while held in the hand (Ex. 31, 32).

If a set of bees has become accustomed to collect honey from artifacts, and paper discs, arranged in pairs of one red and one blue (or discs of any other two colors) are scattered on weeds or placed on weed-high rods, and honey is placed on the discs of one color and none placed on those of the other color, the bees will make regular visits to the color that bears the honey, but will not so respond to the other color (Ex. 8, 9, 10, 11). If these experiments are repeated, using cornucopias (Ex. 19) or boxes with small openings (Ex. 29) in place of the discs, the results will be the same. If bees have become accustomed to collect honey from an artifact of a certain color, and empty artifacts of the same kind and color are placed along side of those that contain honey, many of the bees will enter those artifacts that never have contained honey; empty artifacts of a different color are not responded to in that manner (Ex. 23, 24, 28, 29). After bees have, for a long time, been collecting honey from artifacts of a certain color, if all the artifacts be removed from the field except two that never have contained honey, but one of which is the color of the artifacts from which the bees have been collecting honey, numerous bees will flock into the empty artifact of the same color as those from which the bees have been foraging; but none, or nearly none, will visit the other artifact (Ex. 25, 30). When bees have become accustomed to collecting honey from other sources than flowers, if receptacles of two different colors are placed on a bush, all of one color containing honey and all of the other color being empty, and if, after the bees have been busy for a long time, honey is placed in one of the artifacts of the color that has been empty all along, it will remain on the bush some time before it will be visited by any bees (Ex. 12, 13, 21, 22). All of the facts recorded in this paragraph indicate that the behavior of foraging bees is influenced by colors.

That these bees not only respond to colors, but that they are capable of recognizing them at a distance is evidenced by the

following facts: (1) If, in an open space between their hive and a number of artifacts from which the bees are collecting honey, you place an artifact of the same kind and color and supply it with honey, it will be visited by bees almost immediately (Ex. 18, 29). (2) When bees are collecting honey from red artifacts situated in two different stations, one of which is much nearer the hive than the other and beneath the line of flight of the bees collecting from the more distant station, if all of the artifacts are removed from the more distant station, immediately the number of visitors to the other station is much increased (Ex. 18). This was the case even when the artifact in the nearer station did not contain honey (Ex. 30). Empty artifacts of another color than the honey-bearing color were not responded to in this manner (Ex. 30.).

On leaving one of these artifacts the bee usually made an orienting flight. On the first few visits this was thoroughly done. Facing the artifact and keeping about one centimeter from its surface, she would sidle, in a zigzag line, around the structure two or more times and occasionally reënter it one or more times. Then she would describe one or more spirals, pausing at certain places in the environment as though examining landmarks. In some of the cases I was, apparently, one of the objects thus scrutinized. In harmony with her other behavior, it seems plausible to interpret this as an act by which memory pictures of the environment are formed.

How minute are the details that bees observe I am not prepared to say; but that they do observe details is indicated by the following observations. (1) In the boxes used in the experiments of Series IV., the trays of the boxes used were entered, from the portico, by means of an eccentric opening. In most of the boxes this doorway was rectangular, in others it was circular. When a bee first approached one of these boxes, she had to search for the entrance. After a few trips she would land on the portico directly in front of the entrance, and, in some cases, she would fly into the tray without even pausing on the portico. (2) For about an hour bees had been collecting honey from some red artifacts. It seemed that nearly all of the bees that visited that part of the field were collecting from those artifacts. Alongside

one of the red boxes I placed a red box on the sides and top of which I had pasted bits of white paper. This gave a box with red front and spotted sides. Into this box I placed some honey. The bees that approached this box from the front always entered immediately; the majority of those that approached the sides paused a moment, then went to the nearest red box.

Whether this is a true color vision or simply a greyness discrimination is no easy question to answer; indeed, from our viewpoint, it does not seem an important one. If what to us is red and green appears to the bees as two distinct sensations, as a factor for controlling behavior, it will have the same value to the bee whether it is a red-green discrimination or a grey-grey discrimination. However, the following line of reasoning has led me to believe this a case of true color-vision. Bees that had learned to respond to red boxes in the shadow of the weeds would respond, without hesitation, to similar boxes placed in the sunshine. They responded to the boxes when the sun was shining brightly just as readily as they did when a dark cloud hid the face of the sun. The brightness content of a body in the bright sunlight is quite unlike the brightness content of the same body when in the shadow of weeds; the brightness content of a body in the sunshine is quite unlike the brightness content of the same body beneath a cloudy sky. The only factor common to all of these cases is redness; hence I feel that, with the bees, it is a case of true color vision.

Although odor as a incitive to reflex actions does not play any part in leading bees to flowers, yet odor as a sensation does. If a large number of bees are collecting honey from a cluster of boxes that are all of the same color and you allow the honey of some of those boxes to become practically exhausted while that in the others is constantly replenished, when the workers approach the boxes that are practically exhausted, they, as a rule, do not pass inside; but, pausing momentarily before the box, pass on to one of those with an abundant store of honey. Replenish the empty tray with honey, and the next bee that approaches that box will enter (Ex. 29). This, to my mind, shows that when a bee approaches a box and finds the honey-odor weak she immediately departs for a box where the honey-odor is stronger.

These experiments prove that, to the bee, my colored discs, my colored cornucopias, and my colored boxes were something more than mere sensations; it seems to me that they were true percepts. To the bees those things had acquired a meaning; those strange red things had come to mean "honey-bearers," and those strange green things and strange blue things had come to mean "not-honey-bearers." Hence, whenever the bees saw the red things, they made the appropriate movements for securing the honey, and when they saw the blue things or the green things they passed on. This explains why, in the experiments of series one, discs six centimeters in diameter and well supplied with honey could remain in the presence of hundreds of bees without being responded to by them; and yet, those same bees, a few days later, when those things had acquired a meaning, would enter my red boxes even before I had had an opportunity to attach them to their supports. In their past experience those things had never acquired a meaning, while the small blossoms of the melilotus had come to mean "honey-bearers"; hence they hastened by the feast that had been prepared for them and rushed for the meager supply of nectar in the blossoms of the white sweet clover.

Although Plateau's conclusions are diametrically opposed to the results of this series of investigations, yet the facts related by him are in accord with them.

While proving that bees have color-vision, these experiments throw no light upon the color preferences of insects. That has not been the purpose of these researches. The aim has been to answer the question, Can bees distinguish colors? The experiments seem to demonstrate that foraging bees have percepts and that two factors which enter into those percepts are color sensations and olfactory sensations.

Summer High School, St. Louis, Mo.,
July 18, 1910.

BIOLOGICAL STUDIES ON CORYMORPHA. IV.[1]

Budding and Fission in Heteromorphic Pieces and the Control of Polarity.

HARRY BEAL TORREY.

The large solitary hydroid *Corymorpha* exhibits the phenomenon of heteromorphosis in forms even more striking than those under which it appears in the related *Tubularia*. At the same time, its normal polarity is in several respects more obviously marked. As against a stem, in *Tubularia*, that presents little or no indication of axial differentiation, the column of *Corymorpha* is divided into several regions sharply characterized by differences in form, structure and function. Its diameter varies, being greatest near the base, which is enveloped, for about one third the total length, in a thin layer of perisarc. Beyond the edge of the latter, the naked ectoderm is thicker, its cells are more narrowly columnar, and there is a marked increase in the number of nematocysts. Within the perisarc is the zone of frustules, or rootlets, that form the holdfast and have been homologized with the stolonal processes of *Tubularia*, although they are far more specialized structures. The proximal extremity, conical in form, is furnished with an amœboid ectoderm, by means of which the polyp creeps about.

Not only in structure does one find evidence of regional differentiation, but in capacity for regeneration as well. A hydranth is replaced after section of the column, with a velocity that decreases with the distance from the distal end of the intact hydroid. The differences in velocity are so slight as to be appreciated with difficulty in the distal half of the column, but are easily recognizable in a comparison of rates of regeneration in distal and proximal thirds. Furthermore, heteromorphosis,

[1]Contribution 32 from the Laboratory of the Marine Biological Association of San Diego. Preceding numbers of the Biological Studies on Corymorpha have appeared as follows: I., *C. palma* and Environment, *J. E. Z.*, 1 (1904), p. 395; II., The Development of *C. palma* from the Egg, *Univ. Calif. Publ. Zool.*, 3 (1907), p. 253; III., Regeneration of Hydranth and Holdfast, *ibid.*, 6 (1910), p. 205.

though it may occur after section of the column even below the frustular zone, in the extreme basal region, is most frequent in pieces cut from the column in its distal half.

At the very beginning of my observations on the regeneration of *Corymorpha*, I was struck with two facts: (1) that a segment from the distal half of the column and including the hydranth, does not develop a hydranth at the proximal end until the original hydranth is removed; (2) that when the original hydranth has been removed, the proximal hydranth develops, under normal conditions, more slowly than the distal—another indication, it may be mentioned, in passing, of the initial polarization of the column.

These facts suggested the possibility that, by delaying the development of the distal hydranth on a regenerating piece until the proximal hydranth should have reached an advanced stage of development, the initial polarization might be completely reversed. Accordingly, in the summer of 1902, I performed the following experiment.[1] A segment was cut from the distal half of an average polyp (Fig. 1, *A*). It was then inverted, and the distal cut surface held against the glass bottom of the aquarium by the weight of a steel needle through the distal region (Fig. 1, *B*). The proximal end was free and the stem vertical. At the end of three days, a hydranth flourished at *p*, though there was sign neither of tentacles nor frustules at *d*. The needle was removed. Two days later a small hydranth had appeared at *d* (Fig. 1, *C*), with three distal and four proximal tentacles, somewhat irregularly arranged, probably owing to the wound left by the needle and to other adverse conditions to which that end may have been subjected when pressed against the substratum.

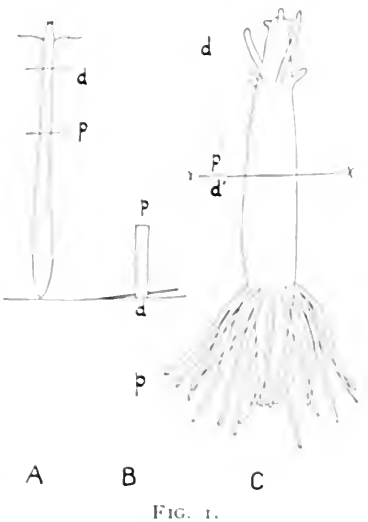

FIG. 1.

[1]All the regenerations noted in this paper occurred under starvation conditions.

It will be noted that while a distal hydranth failed to develop in contact with the substratum, it soon appeared when freed from this contact, in spite of the presence of the large promixal hydranth. On the supposition, however, that the result may not have fully indicated the real state of affairs in the heteromorphic segment, the latter was sectioned at the level x. In two days frustules were appearing at p'; the original polarity of this portion of the column was preserved. But frustules were also appearing at d', and two days later, were unmistakably defined. *In this latter region, therefore, the original polarity was reversed; on a segment of a given polyp, not only had hydranth appeared in the customary position of holdfast, but holdfast had appeared in the customary position of hydranth.*

The outcome of this experiment recalls the reversal of polarity which Loeb later obtained in *Tubularia crocea* when, after accelerating the development of the proximal hydranth by inhibiting the development of the distal, he cut a segment just distal to the proximal hydranth and found that a proximal was now produced more rapidly than a distal hydranth.[1] Morgan and Stevens obtained a similar result on *T. marina* although the polarity of the stem was reversed for but a very short distance from the proximal end in this species.[2]

II.

The suggestion, coming from the above experiment with *Corymorpha*, that section of the column between the hydranths merely disclosed a reversed polarity that already existed but was not, under the conditions, expressed in structural differentiation, led to a number of similar experiments which showed that the original result was in no sense exceptional. I will consider three series of these experiments.

In the first, nine heteromorphic pieces were sectioned at different levels to determine the extent to which each hydranth might control the intermediate region in regeneration. In no. 1, the distal hydranth was removed by a cut immediately below it

[1] *Pflüger's Arch.*, 102 (1904), p. 152; trans. in *Univ. Calif. Publ. Physiol.*, 1 (1904), p. 151.

[2] *J. E. Z.*, 1 (1904), p. 559.

(Fig. 2, *a*); it was replaced by another hydranth that at the end of ten days was in the condition shown in Fig. 2, *b*. No. 2 was a similar case. In neither of these cases was reversal exhibited. No. 3 died. No. 4 is represented in Fig. 3, the proximal hydranth being much less developed than the distal, and the plane of section passing near but not immediately distal to it. In four days both

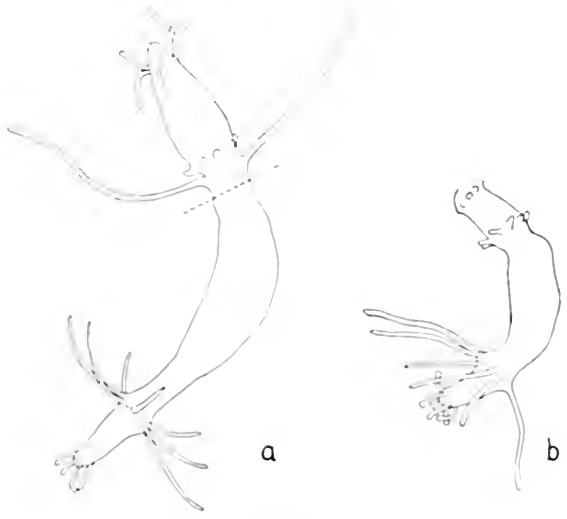

FIG. 2.

segments possessed frustules; the proximal segment had, accordingly, reversed completely. No. 5 (Fig. 4) exhibited another case of complete reversal in the proximal segment; both segments were as shown in Fig. 5 at the end of five days. No. 6 was a similar case. No. 7, cut when in the condition shown in Fig. 6, appeared, five days later, as shown in Fig. 7. No. 8, a similar case, exhibited similarly a complete reversal in five days. No. 9 resembled Fig. 2, *a*; but it was the smaller, *proximal* hydranth that was removed, by a cut immediately distal to it; in seven days a new hydranth was established in its place.

According to these results, heteromorphic pieces produce holdfasts at the wound when sectioned approximately midway between the hydranths, but produce hydranths at the wound on the longer pieces in those cases in which either hydranth has been removed by a cut immediately below it.

The second series of experiments involved 14 heteromorphic pieces whose proportions and stage of development are represented in the accompanying diagrams (Fig. 8). These pieces were cut as indicated in the diagrams. Four days later, both parts of *a*, *c*,

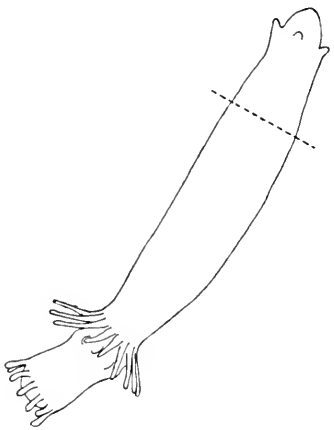

FIG. 3.

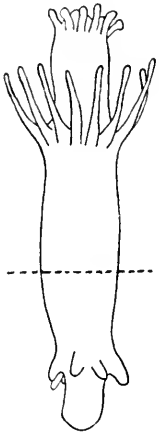

FIG. 4.

d, *e*, *f*, *h*, *i*, *l*, were attached and possessed frustules; the polarity of one of the pieces in each of these cases, accordingly, was completely reversed. At the same time, both portions of *b* were

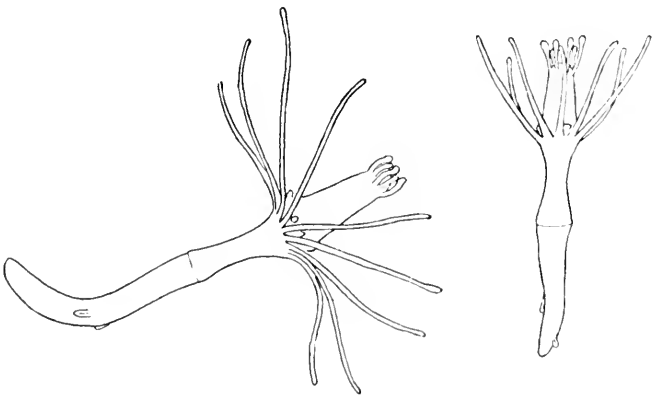

FIG. 5.

regenerating as single polyps, one being attached and furnished with frustules, the other being unattached and lacking frustules. Under *g*, four heteromorphic pieces were grouped. Four days

after cutting, all were regenerating as single polyps, six being attached and possessing frustules, two being unattached and lacking frustules. Both pieces of *k* were attached, three days after

FIG. 6.

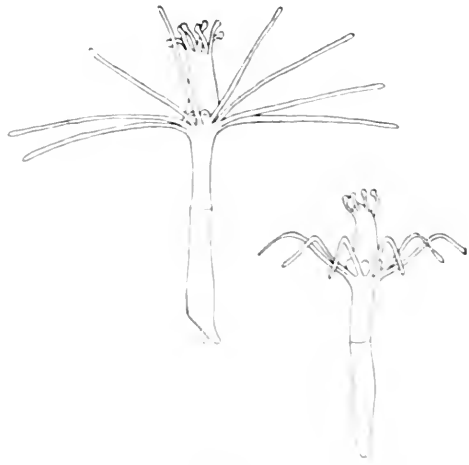

FIG. 7.

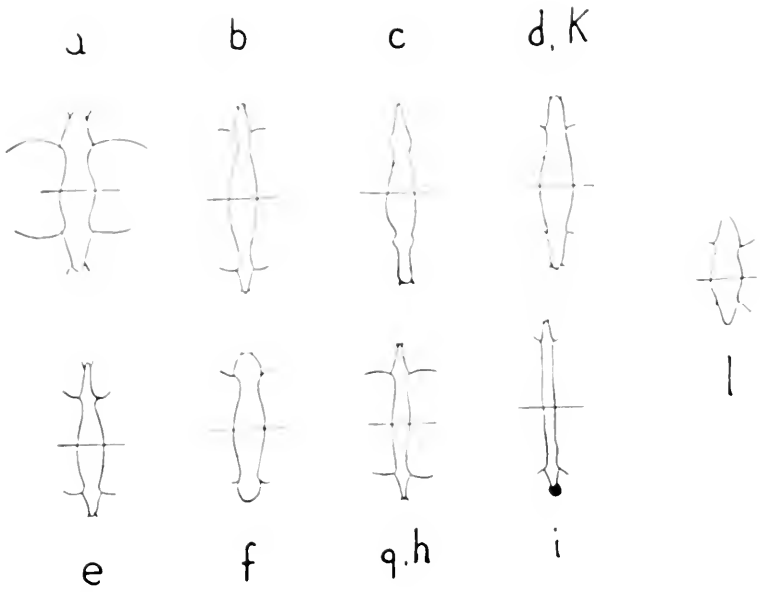

FIG. 8.

cutting, but owing to accidental neglect, disintegrated before forming frustules.

It should be noticed especially that all the heteromorphic pieces used in this series were *short*, and none were above medium diameter. *In not a single case, under these conditions, did the polarity fail to reverse in one of the portions into which the heteromorphic pieces were divided.*

The third series shows the importance of this consideration of length. Three heteromorphic pieces, absolutely and relatively much longer than those of the second series, one of them considerably larger than the other two, were sectioned as shown in Fig. 9. Five days after section, all six pieces were heteromorphic which indicated that in none of them, whether distal or proximal, was development at the wound dominated by the conditions, existing at the other end of the piece.

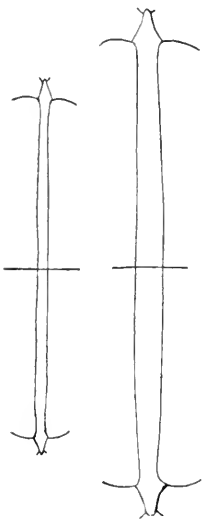

FIG. 9.

The same fact is brought into clear relief by a comparison of the following figures. Of 14 segments representing the distal half of the column of 14 polyps of moderate size, 12 were heteromorphic in 3 days. Of 81 very short segments from several small polyps, only 6, or 7.4 per cent., became heteromorphic. Further, of 13 segments of approximately the same length and diameter as the pieces obtained by cutting the heteromorphic pieces in series 2, 8 became heteromorphic. Of 15 similar segments, 10 became heteromorphic.

Besides these figures, there is a mass of evidence, obtained by repeated experiments on large numbers of individuals, demonstrating that the presence of the original hydranth on a segment of the column inhibits the development of a proximal hydranth.

It is clear, then, in the light of the facts cited in this section, (1) that *reversals of polarity profound enough to effect entire segments of the column as units are readily produced in Corymorpha;* and (2) that *the stage of differentiation at one end of a piece will under certain conditions control differentiation at the other end.* That reversals of polarity, in cases of heteromorphosis, are often shown, by form changes, to affect considerable areas of the column, without aid from the knife, will appear in the following section.

III.

As the first evidence in this direction I may refer at once to certain U-shaped figures formed from heteromorphic segments by the attachment of the latter to the bottom of the aquarium dish by means of adhesive ectoderm developed on one side of the column. Such a case is shown in Fig. 10, which represents the condition of a segment sixteen days after its removal from the distal half of a column. A constriction defines the aboral limits of the regenerating polyps. Frustules are present in two groups,

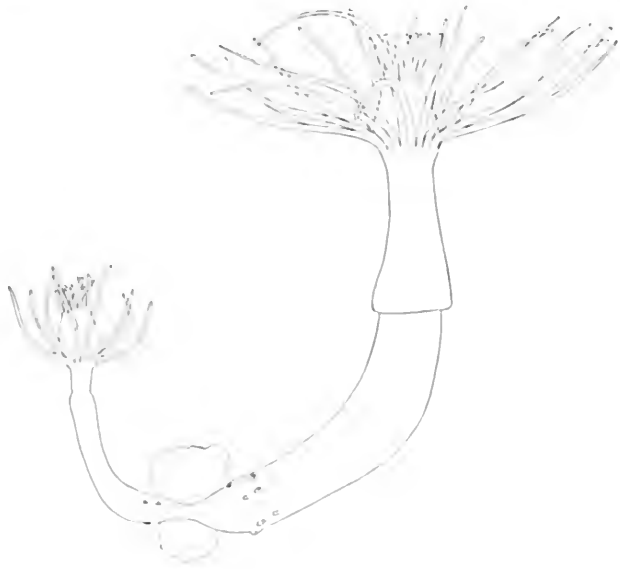

FIG. 10.

roughly proportional in numbers to the sizes of the polyps to which they belong, and situated in the position normally occupied by frustules in the adult. The smaller polyp approaches more closely to the proportions of the larva. On the following day, this process of fission had been completed, probably by rupture, although the proximal ends of the two polyps were then rounded and smooth without traces of such a process.

Through my failure, after repeated attempts, to obtain many cases of fission of this sort, I found that a necessary element in the process was the adherence of the heteromorphic piece to the

substratum. I pointed out some years ago[1] that *Corymorpha* is negatively geotropic, even small fractions of the column reacting with great definiteness. This tendency to bend away from the center of the earth can be effective, however, only when the reacting piece is properly anchored. Pieces free from the

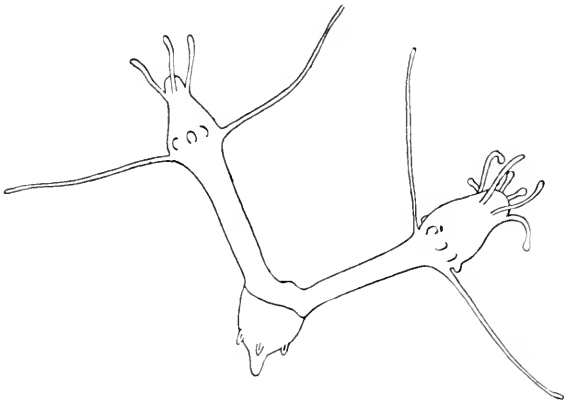

FIG. 11.

substratum never exhibit the reaction. As soon as they are attached, it appears; and U- and Y-shaped figures are formed when pieces are heteromorphic and the point of attachment is between the developing ends.

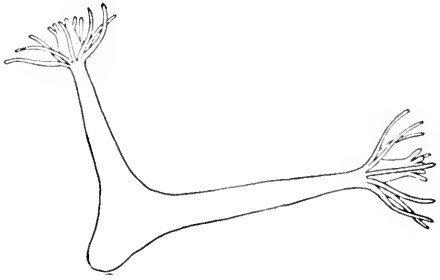

FIG. 12.

Each Y-shaped figure is formed from a straight piece by the development of a protuberance that corresponds to the stem of the Y, and is terminally adhesive (Figs. 11, 12, in both of which the limbs of the Y have become much attenuated during the

[1] *J. E. Z.*, 1 (1904), p. 395; *Univ. Calif. Publ. Zool.*, 2 (1905), p. 335.

development). Such protuberances and Y-shaped figures are found in nature only in the most exceptional cases, where they probably arise in response to the same conditions that bring them forth in the laboratory. To present these conditions with sufficient fullness, I must refer briefly to other abnormal forms that have often appeared in neglected aquaria.

It was often difficult, in warm weather, in the absence of running water, to prevent the growth of bacteria in the dishes in which freshly collected polyps were placed. Under the influence of fermentative changes induced by these conditions, the hydranths would cast their large proximal tentacles and medusiferous peduncles, the distal tentacles would be absorbed, and the column would come rapidly to be surmounted by a more or less rounded mass of tissue that might show two or three knobby irregularities. The columns were affected in a much less degree if at all. In fact it is quite easy to avoid these difficulties altogether by removing the hydranths as soon as the polyps are collected. This fact is doubtless due to the relatively large mass of protoplasm in, and the greater differentiation of the tissues of the hydranths.

Upon the reduction of the hydranths to the knobby masses just mentioned, and the removal of the débris composed of disintegrating tentacles and medusæ with their peduncles, the bacteria would disappear, fermentative processes would lead to the substitution, for the original hydranths, of various monstrous forms—double hydranths, hydranths with double or triple probosces in varying degrees of independence, combinations of hydranths with varying numbers of probosces, etc. These phenomena indicated the breaking up of the original single physiological system into several, the first sign of this multiplication appearing in the irregular form of the terminal mass of tissue. Each irregularity was the center of a budding process. And without laying any emphasis on the manner of its initiation, each budding process may be compared directly with the process by which the stem is produced in the Y-shaped figures we have been considering.

In one important respect these budding processes resemble each other; they stand, namely, for a certain disorganization (how

produced may, for the time, not detain us) in the original physiological system. In another respect they differ, in that they lead, in the one case, to a hydranth, or part of a hydranth, in the other to a holdfast. This difference is essentially an expression of the different conditions controlling their development, of which the influence of adjacent parts is the chief. The intimacy of this cöordination is obviously a function of the physiological isolation of the parts concerned. It is a conspicuous fact that Y figures are formed almost invariably from short heteromorphic segments; shrunken, starving, slow developing (Fig. 13, which shows eth beginning of a bud) pieces give an especially large proportion of them. Frequently that portion of a segment of the column which lies against the floor of the aquarium puts forth tentacles more slowly than the upper surface. This retardation in development, due probably to diminished supply of oxygen next the substratum, is accompanied by a bending of the segment by means of a contraction of the affected side. It is on the opposite, convex, aspect of the column that the bud develops (Fig. 14, in which tentacles and gonads are only partly drawn), not, therefore, in direct contact with the substratum. And it is significant that, with the rarest exceptions that are referable to exceptional con-

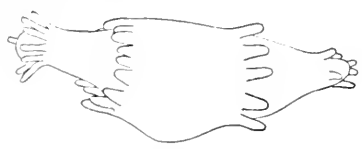

FIG. 13.

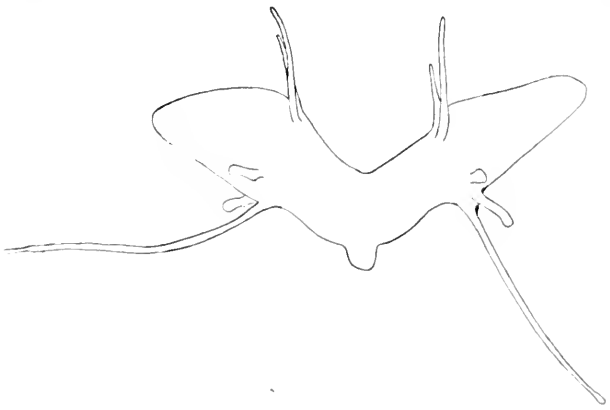

FIG. 14.

ditions, such buds arising on heteromorphic pieces become holdfasts.

These facts indicate that while the origin of the bud depends upon a degree of disorganization in the original physiological system, its *fate* depends upon a secondary physiological coördination with the hydranths between which it develops. The bud acquires the distinctive character of a holdfast, namely, its adhesiveness, independent of any influence of the substratum. It has been pointed out already that a hydranth at one end of a piece exercises a profound influence upon the differentiation that may occur at the other end, depending on its own stage of development and its distance from that end. In the Y figures, then, there is a developing region between the two hydranths whose differentiation is controlled to some extent by them. An interesting case of the coördination of parts in a short piece developing as a Y figure, is represented in Fig. 15. A new axis at right angles to the original axis has been established. And the orientation of gonads and tentacles—especially the latter—is clearly a resultant of the redistribution of forces correlated with that change.

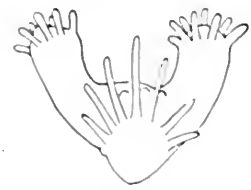

FIG. 15.

Fission may be considered as a special case of budding, depending upon the length of the heteromorphic piece involved. As the piece lengthens the tendency to bud vanishes, until the characteristic of adhesiveness alone remains. This indicates that the region between the two hydranths is still controlled by them, while the constriction and the frustules on either side of it (Fig. 10) mark the increasing effectiveness of their independence—or of the systems of which they form important parts.

It is only on heteromorphic pieces of moderate length, however, that fission of this type has been observed. Just as it was from relatively short heteromorphic pieces only that the reversals described in the previous section were obtained, the proximal elements of the longer pieces failing to produce holdfasts at the wound, so it has been only in the shorter heteromorphic pieces that the column between the hydranths has shown any signs of budding, constriction or frustule formation.

The longer pieces failed to differentiate in these directions, although attempts were made to encourage such developments, as follows:

1. Nine long heteromorphic segments were held against the aquarium bottom by weighted glass needles laid across their middle. One was cut in two in two days; neither portion had developed frustules twenty-four hours later. One was cut in two in three days; both portions had aboral processes, but no frustules developed in the following twenty-four hours. Four escaped from their needles on the third day, and showed no change at the end of another day. One was almost cut through by the needle, and three processes were formed at the wound, but no frustules or adhesiveness at the end of another day. One remained under the needle unchanged for four days, when the experiment terminated with my departure from the laboratory.

2. Discontinuity was produced by ligature on 16 long heteromorphic pieces two days after regeneration had begun. In two days, 3 had broken into two parts. Two days later, 6 more had done so; the next day, 7 more. Seven days after the ligatures were applied, 2 had frustules at the ligatures. Both were contracted and opaque—signs of structural degradation; 3 possessed no frustules at the ligature; on the contrary, the proximal segment of one possessed a hydranth there. All the other ligatured pieces had separated into two portions, 18 in all, of which but 4 were attached aborally and possessed frustules.

The first of these experiments serves to emphasize the feebleness of contact as a formative influence, while the second adds to the evidence that differentiation in the region between heteromorphic hydranths depends in an important degree upon the distance between them, which other things equal, is an index of their control.

That the longer pieces do not show signs of fission, then, is to be attributed, I believe, to the freedom of the intermediate region in each case from the effective control of the physiological systems on either side, that may be conceived as extending over it from opposite directions. Where these systems are near enough together, a new compound system is created, of which a bud may form a part. When farther apart, their disharmony may appear in the phenomnea of fiisson.

These statements are obviously very general. Since all short heteromorphic pieces do not either bud or divide by process of fission, there must be a factor still undefined that determines the lack of uniformity. There is no doubt that external agencies can facilitate either budding or fission. Small wounds in the side of the column may lead to a variety of results, including sporadic tentacles, and buds furnished with tentacles or frustules, or neither. In this connection two heteromophic pieces, cut in the middle region, half way through the column, gave the following results. On the longer piece, a narrow bud developed in four days at the wound, with neither frustules nor tentacles. On the shorter piece, frustules developed around a blunter bud at the wound, before attachment took place. In this case, the wound was sufficient to break up the original system existing at that point and initiate a new development. The fact that frustules appeared shows the control of both hydranths on that development.

Budding occurs, however, when there is no sign of local injury from without. And it is difficult to account on this ground alone for the fact that the large majority of buds arise approximately midway between the hydranths. To my mind, far more significant is the fact that buds develop so often on pieces obviously in poor physiological condition *generally*. It is then that the physiological continuity of the piece through the transitional middle region might be expected to be especially affected by disruptive tendencies springing from the antagonism of proximal and distal systems—so obviously antagonistic in the fission shown in Fig. 10. In that case, the canals are completely obliterated at the constriction and the tissue is opaque and apparently impoverished. This constricted region is under tension, which probably accounts in part for its form. The tension is produced by the active migration of the two polyps away from each other, in the manner of the opposite halves of an anemone in process of fission by rupture.[1] The initial discontinuity is thus accentuated by the activities of the polyps themselves.

[1] Torrey, *Univ. Calif. Publ. Zool.*, 1 (1904), p. 211.

IV.

Discontinuity can be established experimentally not only by the knife, which entails a wound, but, as already indicated, by ligature, by the use of which a wound can be avoided and conditions obtained that more nearly approximate those described in the last section.

This method has been used on *Tubularia* by Driesch, Morgan, Loeb, and Morgan and Stevens, and results obtained which are of interest in the present connection. By ligating segments of the stem, not only is the production of aboral (proximal) hydranths assured, but accelerated; and only exceptionally, after much longer periods, is there any development at the ligature itself. Loeb succeeded in showing that the acceleration of the development of the aboral hydranth is an indication of reversed polarity that exhibits a certain stability in regeneration. This is in accord with what I had already observed in *Corymorpha*, where reversals of polarity accomplished without the aid of the ligature are even more marked.

The experiments with ligatures have been repeated so many times on *Tubularia*, that it is hardly necessary for me to refer at present to similar experiments of my own farther than to say that the ligature accelerated the development of the aboral but not of the oral hydranth, and in no case was there any development at the ligature, on either side of it.

In Corymorpha, as in Tubularia, ligatures accelerate the velocity of development at the proximal ends of segments of the column. The fact does not stand out with such dramatic clearness, however, partly because there is greater individual variation in rate of regeneration, partly because the time intervening between the appearance of distal and proximal hydranths is much shorter. That such an acceleration occurs can be shown by an experiment like the following: Segments about 2 cm. long were cut from the distal half of 20 polyps, a ligature being passed tightly around each near its distal end. Segments of similar length were cut from the distal halves of 21 polyps of similar size; these were not ligatured. All were placed together in the same dish. In 28 hours, there were signs of proximal hydranths on 13 ligatured segments, and 14 on non-ligatured segments. The condition of

affairs at the end of 56 hours is shown in the following table, in which the serial numbers represent stages in the development arbitrarily selected for purposes of classification. Under each of these appears the number (1) of proximal hydranths in that stage of development on the ligatured segments, (2) of proximal hydranths on non-ligatured segments, and (3) of distal hydranths on non-ligatured segments.

TABLE I.

Dev. stages	1	2	3	4	5	6	7	Totals.
1. Lig. (pr.)	0	5	3	5	5	1	1	20
2. Non-lig. (pr.)	4	4	5	6	1	1	0	21
3. " (dist.)	3	2	8	4	1	2	1	21

In spite of the individual variation represented by these figures, they *show a tendency in the proximal ends to develop hydranths somewhat more rapidly on the ligatured segments, and almost as rapidly on the latter as the distal hydranths on non-ligatured segments.*

V.

Although *Corymorpha* responds proximally like *Tubularia* when segments of the column are ligatured as above, there is an important difference in its response at the ligature, namely, in the rapidity with which hydranths are formed immediately below it. In *Tubularia*, a hydranth very rarely appears immediately below the ligature, and then only after the lapse of many days. In *Corymorpha*, on the contrary, hydranths form readily and frequently in this position.

FIG. 16.

That a hydranth should arise immediately below a ligature in *Corymorpha* might be anticipated from the occasional occurrence of such monsters as that shown in Fig. 16, which represents a regenerating segment of the column. The proximal hydranth (below the angle) is not so far along as

the distal hydranth, and has apparently developed later, below an interruption in the physiological continuity of the column comparable with what a ligature might produce. In fact, just such cases have been produced experimentally several times. A typical experiment may be recorded, showing incidentally the difficulties that made the number of positive cases so small.

The column of *Corymorpha* is very mobile, capable of considerable changes in length and bulk, and its tissues are very delicate and easily ruptured. So it has been difficult to make ligatures tight enough to interrupt the currents in the canals, as well as possible diffusions through the axial cells, without so weakening the column as to lead to complete rupture in two or three days. This has been accomplished, however, in a number of cases sufficient for the present purpose.

Experiment 1.

April 29, 1910, 3.30 P. M. Sectioned 20 polyps of similar size, about midway of the column, and ligated each just below wound, leaving a small segment of tissue above the ligature.

May 1, 9.30 A. M. Four stumps removed, ligature having come away with terminal button of tissue.

May 2, 5 P. M. Thirteen more removed for similar reason. There are hydranths on these stumps that seem to be too far along, under the conditions, for 31½ hours (*i. e.*, assuming the separation to have occurred immediately after the previous survey of them, which is not probable).

Of the remaining 3, 2 show nothing below the ligature, while the third appears as in the semi-diagrammatic Fig. 17, which bears a striking resemblance to Fig. 16.

May 3, 9.45 A. M. The two hydranths of Fig. 17 have fallen apart.

May 4, 10.00 A. M. Of the 2 stumps showing no development below the ligature of May 2, one (*a*) has now budded a set of tentacles just below the ligature; the other (*b*) as before.

May 6. The tentacles of (*a*) are larger.

May 9. Still no change in (*b*). Exp. abandoned.

In a second experiment, 6 columns were tied as indicated in the diagram (Fig. 18, *a*), after removal of hydranth.

EXPERIMENT 2.

April 7, 3.00 P. M. Hydranths removed and columns tied.

April 8, 1.00 P. M. (*a*) Distal ligature and tip fragment (1) broken away in 2 cases. (*b*) The three segments have separated in 1 case. (*c*) 3 cases in original condition.

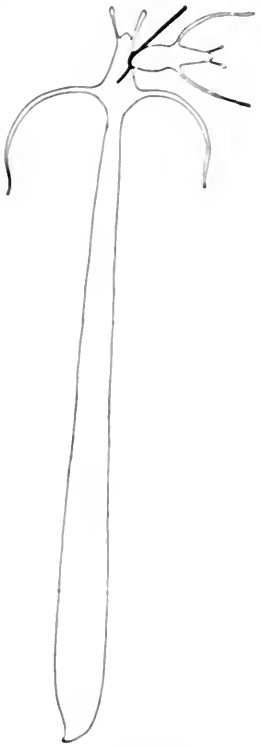

FIG. 17.

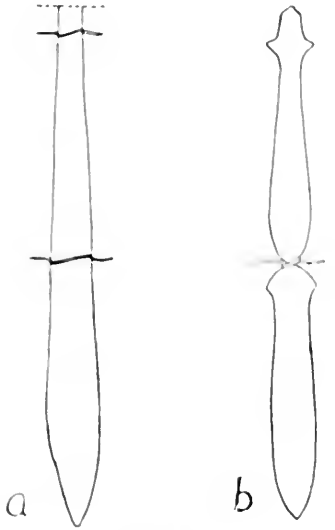

FIG. 18.

April 9, 2.00 P. M. (*a*) First case. Distal hydranth appearing on seg. 2, also on seg. 3 but not so far along. Second case. Distal hydranth appearing on seg. 2; not apparent on seg. 3, (*b*) —. (*c*) First case. The segments have separated while under observation. There are signs of a very slight rupture, and indications of tentacles on seg. 2 distally. Second case. The segments separated at 10.00 A. M. Seg. 2 shows signs of tentacles at both ends. In seg. 3, tentacles are being shadowed forth distally. Third case. The segments have separated,

seg. 2 showing faint signs of tentacles distally; seg. 3 in poor shape, removed.

April 10, 9.00 A. M. (*a*) First case. See Fig. 18, *b*. Note tentacles appearing just below ligature. Second case. Similar to first case; tentacles on seg. 3 not quite so far advanced.

April 11, 2.15 P. M. (*a*) Segments in both cases broken apart; exp. abandoned.

Of the six cases considered in this experiment, three show development of hydranths immediately below the ligature.

In a third experiment, the hydranth was removed from a polyp and the column ligatured near the cut and near the base. In three days, tentacles had budded just proximal to the distal ligature, as well as on the small distal segment. The next day, the latter was loosely joined to the segment proximal to it; both segments possessed hydranths with both sets of tentacles.

These results show that *hydranths form readily immediately below the ligature* in experiments like the foregoing.

VI.

The failure of *Tubularia* in such experiments to form a hydranth immediately below the ligature with the facility exhibited by *Corymorpha* is correlated with an important structural difference. The stem of *Tubularia* is encased in a stiff, chitinous layer of perisarc, that offers a certain barrier to the diffusion of gases between cœnosarc and the surrounding medium. The column of *Corymorpha*, furnished with a thin, rudimentary perisarc about its base, is naked for more than half its distal length; in this naked distal region, the ligatures were in all cases located.

When a ligature is passed tightly around a stem of *Tubularia*, the cœnosarc is not only ruptured, but the perisarc, itself intact in most cases, is drawn closely about each end of the cœnosarc thus produced. The result is that, while discontinuity has been established, the cœnosarc remains, as before, separated by the perisarc from the surrounding medium. When a ligature is passed tightly around a column of *Corymorpha*, discontinuity is established without in any way interfering in a comparable degree, if at all, with the diffusion of gases between cœnosarc and sea water. Now, when it is remembered that a discontinuity

brought about by a knife in *Tubularia* leads promptly to regeneration at the wound, the fact suggests that *Tubularia* develops with greater difficulty than *Cormyorpha* at the ligature because the cœnosarc receives a smaller supply of oxygen in that region.

This view was tested by the following experiment, in which the factor of contact, which tends to inhibit development orally in *Corymorpha*, was eliminated.

Fifteen pieces, each about 2 cm. long, were cut from fifteen *Tubularia* stems of similar size and condition, just below the hydranth. The distal end of each was inserted in a capillary glass tube (closed at one end by a paraffine plug) into which it fitted easily, without terminal contact, *except with a small quantity of sea water;* the proximal end was free. As a control, 16 similar pieces were cut, both ends remaining free.

Forty-five hours later, the pieces in the tubes had developed nine hydranths on the outer (proximal) ends, nothing on the inner ends. In the control, though fifteen of the sixteen pieces possessed distal hydranths, no proximal hydranths were visible.

Twenty-five hours later still, all the pieces in the tubes possessed proximal hydranths; nothing had developed on the inner (distal) ends. In the control, only eight proximal hydranths were present.

Removed now from the tubes, all the pieces rapidly produced normal hydranths distally.

This experiment seems to establish (1) *that the failure of Tubularia stems to form hydranths, when ligatured, immediately below the ligature, is due to lack of oxygen;* (2) *that encasing the cut distal end of a stem in a glass cap leads to the same acceleration of development of the proximal hydranth as does the presence of a ligature;* and (3) *that accordingly, such acceleration is due to the inhibition of the development of the distal hydranth in the absence of an adequate supply of oxygen, rather than to an interruption of or other change in the course of the circulation in the canals.* The circulation is merely a transportation system, carrying substances favoring development that are removed from the stream by those tissues especially that have free access to oxygen, that is, by the tissues at the open ends of the perisarcal tube. The possibility that oxygen might play such a role in this process of selection

was suggested by Loeb,[1] though in a somewhat different form in connection with a discussion of the function of the red pigment granules of the circulation.

The acceleration of proximal development in ligated pieces of *Corymorpha* is connected, not so much with any effect the ligature may have upon the supply of oxygen to the neighboring tissues, as with the inhibitory factors of contact and necrotic change that it introduces. The same factors may play a certain part in the inhibitory effect of the ligature in *Tubularia* also.

It will be remembered that if a piece of *Tubularia* or *Corymorpha* is ligated in the middle, development at the distal wound does not exhibit the acceleration characteristic of development at the proximal wound. This difference is to be explained, I believe, in the following way. Inhibition of development at the ligature on its proximal side hinders the utilization of a certain quantity of substances that would be used up proximally were the ligature not present. That the availability of this material for the distal end does not obviously accelerate the development distally is due, probably, to the initial acceleration of the distal over the proximal development in the absence of the ligature, that is, under conditions of active competition with the proximal end.

VII.

The considerations in the foregoing sections lead to the conclusion that the polarity of *Corymorpha*, of which the initial acceleration just mentioned is one expression, is a product of conditions under which the organism develops, changing as they change; that it is essentially but an inclusive designation for certain phenomena that depend upon both internal and external conditions, all of which can be experimentally controlled. The internal conditions appear in the effect of the continuity of tissue in an intact stem, and the presence of the original hydranth on a segment of stem, both inhibiting the development of a hydranth. The external conditions are represented by oxygen, contact, and necrotic changes such as are produced by ligatures. The first is necessary to all development while the others inhibit the development of the hydranth.

[1] *Loc. cit.*

With the exception of the third, these are conditions that govern the development, whether embryonic or regenerative, and they are just the conditions by whose manipulation the original polarity of the column can be reversed. Since such reversal depends upon an acceleration of proximal relative to distal development, it would seem reasonable to suspect that a local acceleration in the development of the embryo might be the efficient cause of the initial polarity in the individual.

Such a local growth area appears in the embryo at the point which first leaves the egg case and thereupon defines the oral extremity.[1] This extremity may emerge at any point not adherent to the substratum; which qualification indicates that contact limits to a considerable extent the area in which the oral pole must appear. Since the egg case is a certain barrier to the diffusion of oxygen into the egg it is possible that variation in its thickness may be an important factor in determining the position of the oral pole in this area.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CALIFORNIA,
August 9, 1910

[1] Torrey, *Univ. Calif. Publ. Zool.*, 3 (1907), p. 259.

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BIOLOGICAL BULLETIN

BIOLOGY OF THE SHAWNEE CAVE SPIDERS.

NORMAN E. McINDOO.

General Introduction.

From September 7, 1908, to September 7, 1909, the author held the Speleological Fellowship in Indiana University with residence at the University's Cave Farm three miles east of Mitchell, Ind. The present paper embodies the results of the observations during this time on the habits of spiders in caves and in the laboratory.

The work has been carried on under the direction of Dr. C. H. Eigenmann, professor of zoölogy in Indiana University, to whom I am indebted for suggestions and the loan of his cave literature. I wish to express my thanks to Dr. Charles Zeleny, associate professor of zoölogy, for many helpful suggestions. I am indebted to Dr. Alexander Petrunkevitch for the identification of the specimens; to Dr. A. M. Banta, for the loan of his entire collection of cave spiders, and to Mr. Will Scott, for part of the map of this series of caves. He surveyed and mapped the cave from "1" to "37" or the Upper Dalton (see map, page 321) in the autumn of 1907; the author assisted by Mr. Frank Green surveyed and mapped the Upper Dalton from "37" to "64" in October, 1908.

Previous Work.

Published observations on the habits of cave spiders are limited to a few scattered papers which give lists of species and localities.[1] The best studies published are those of Packard[2] and Banta.[3]

[1]Banks ('06) and Blatchley ('96) give lists of Arachnids from Indiana Caves.
[2]*Mem. Nat. Acad. Sci.*, IV., 1888.
[3]"Fauna of Mayfield's Cave," 1907.

The Species Studied.

Two species of spiders permanently live in the Mitchell caves. One, *Troglohyphantes* (*Willibalda*) *cavernicola* Keyserling, a linyphid[1] is a true cave form; the other, *Meta menardi* Latreille, is an epeiridid and also lives outside of caves. According to Emerton (1902, 190–) the latter arachnid "lives in caves and similar cool and shady places in various parts of this country and also in Europe."

Banta (1907, 65) reports *Erigone infernalis* Key. from the Twin Cave at Mitchell, Ind. I have been unable to find it here, but have taken it in Mayfield's Cave at Bloomington, Ind.

Troglohyphantes has been observed in detail in order to get as far as possible the life history of a typical cave spider. The distribution, food and results of the experiments of *Meta* are given in order to show how an outside form is able to adapt itself to a subterranean life. All notes unless otherwise stated refer to *Troglohyphantes*. The numbers in quotation marks refer to localities in the caves (see maps, pages 321 and 323).

In *Troglohyphantes* there are all degrees of differences in coloration, and in the degeneration of the eyes. The abdomen varies in color from black, dark brown, light to white. The cephalothorax varies from dark, pinkish, light to a white color. The most common combination of colors is a light brown abdomen with a pinkish cephalothorax. The adult females range in length (cephalothorax and abdomen) from 2.4 mm. to 3.7 mm., while the adult males vary from 2.2 mm. to 3 mm.

In the adults the eyes range from eight in number, each with a maximum size of 0.036 mm. in diameter to no external signs of eyes. I have not seen Keyserling's description, but from his figure, which Packard[2] has copied, the eyes are small, and the front middle ones extremely minute.

Physical Environment.

These spiders are found only in total darkness, where the atmosphere is saturated, and in places suitable for the construction of snares. They are never found where the walls are perpendicular with water covering the entire floor; nor are they

[1]Banks, '06, classification.

[2]1888, Plate XV., Fig. 32.

found where the walls and floor are dry. Distance from the entrance does not necessarily limit the distribution if the three necessary conditions are present, nor does scarcity of food limit their distribution to a very great degree.

At "2" a few were collected last fall but none has been found since. This is 200 feet from the entrance in total darkness. At "9" a few more than at "2" have been found; at "10" a great many have been taken and "13" was my best collecting ground. This place is 600 feet from the mouth. On several occasions I have gathered two or three dozen individuals here and such wholesale collecting at one place seemed at the time to exhaust the supply, but a month after such a collection had been made, I have been able to duplicate this record. Many have been collected at "14" and at "19." The latter locality is the "Big Room," 1,700 feet from the entrance. Three were seen at "23" and only a few were observed in a branch at "31." This latter place is in total darkness 200 feet from the mouth of the Lower Twin Cave at "32." Not one has been observed between "33" and "36." A few have been collected in a branch at "38" in the Upper Dalton. This location is in total darkness 150 feet from the entrance. A number were taken from branches at "46" which is 1,024 feet from the entrance. This locality was my second best collecting ground, particularly for the cocoons. A few were taken at "50." One was caught in the "Big Room" at "57" and several snares were seen. At "63" almost a mile from the entrance of Upper Dalton, three were observed and several webs were among the rocks at a "cave-in."

Troglohyphantes is also quite abundant in Hamer's Cave one half mile west of these caves. None was found nearer than 300 feet from the entrance.

This archnid shares as wide a distribution as the blind beetle, *Anophthalmus tenus* Horn (Blatchley, '96). It far outnumbers the latter in individuals, but is less evenly distributed. By actual count there are twice as many females as males in all the collections made from the various caves.

Meta is found both in twilight and total darkness. In the Shawnee Cave they are quite abundant from the entrance at "1" to "4." The latter place is over 200 feet from the entrance

in total darkness. Several were seen at "6," 100 feet from the mouth. An adult male was observed on the roof at "14"; nine days later, it was seen under a rock on the floor and had constructed a snare. One immature specimen was seen in a branch at "23." At "30," 200 feet from the entrance of Lower Twin, a few live, and at "32" and "33" they are quite numerous.

May 17, I collected eighteen *Metas* and placed them on the north wall by a large pile of rocks at "19" in the "Big Room." August 3, I saw six of them. They had scattered along the wall about 50 feet and among the rock pile. Each had built an orb-web and remains of small diptera were seen in the snares. August 17, after a brief search I saw only three of the eighteen. This absence does not mean that they had died, but that it was impossible to find them.

Locomotion.

This arachnid is very agile and is a good runner. Its long legs and slender body enable it to move from place to place, and to avoid an obstacle with much ease. When not irritated it moves along slowly and gently. When stimulated mechanically, it gives a quick jump, runs and dodges whatever obstacle may be in its way. However, it runs only a few inches and if stimulated a second or third time often drops in an instant and hangs to its web. Sometimes it climbs the web to the place from which it dropped, at other times it lets itself down to the floor and then runs off.

Several were placed on the roof among beads of water and other small obstacles. A pencil was used to stimulate and guide them so that they were obliged to run up against the obstacles. They use the first pair of legs as feelers. These are kept well in advance of the other legs and head so that they can detect an object in front of them the length of the body. While walking or running slowly, they are able to dodge an object every time. If caused to run swiftly, they run against the object, or if the object be a low bead of water, they run over it and pass on.

Webs and Snare.

This spider usually spins a web wherever it goes. It is impossible to see a single thread in the cave with a carbide bicycle

lamp unless the thread is coated with water. Quite often on the roof in a slit between two strata of rocks, or in crevices in the walls are found collections of webs which are generally coated with water. These webs do not seem to be of any service to the spider after once spun. They, however, show how it wanders from place to place. It makes a flat sheet-like snare under which it lives. The snare is slightly curved downward and may lie in such a position as to form any angle between 0° and 90° with the floor. The threads are fine and the snare so transparent that it is difficult to see unless it is coated with water. The meshes are so minute that the snare turns water. The snare is supported by many fine threads from the sides, the length of these vary according to the surroundings. When insects fly into the snare they are taken through it by the spider which is on the under side.

Snares are most abundant in the older parts of the caves where the passages are blocked up with clay. At such places the clay banks are more or less perpendicular and the water has washed out many small crevices. Sometimes large blocks of clay tumble down into a heap. Among these heaps of clay blocks and in such crevices as the above the snares are very numerous. In a crevice five inches wide I once saw three snares built one above the other not more than three inches apart. All three were parallel with the floor.

In several places snares are quite conspicuous in the angle between the floor and wall. In such positions the spiders have better routes for travel from one snare to another than in the clay banks.

The third place where snares are found is among the rocks at "cave-ins." The rough cornered rocks appear to form good places for attachment and quite frequently dripping water is present which this species apparently enjoys.

Food.

While in captivity I have fed them small gnats, small flies and the spiderlings of *Meta*.

In the caves the following observations were made: At "13" while watching a male who was trying to court a female I saw

a white-winged dipteron fly into the snare. In an instant the female seized the insect, then ran back to the male. The latter then ran to the other side of the snare. In a few minutes another white-winged dipteron flew into the snare near the male, and he lost no time in seizing it. Several other times these diptera flew against the snare but failed to be entangled in its meshes. In each case the spiders made a dash for the would-be victim while holding one insect in their mandibles. At an earlier date a spider at this same place was seen eating a white-winged dipteron. On various dates at "14" one was caught eating a myriopod; one, a small gnat; and several were caught under an old turtle's shell with thysanurans.[1] At "19" two or three were observed in a mouse trap with some cheese. At "40" one was caught under an old piece of meat with a small white thysanuran in its mandibles. At "43" one was eating a white-winged dipteron, this spider was running on the wall. At "50" one was seen in its snare eating a small myriopod.

This spider is usually very peaceful. Neither in the caves nor in captivity have I ever seen them to show the least signs of pugnacity when they came in contact with each other. Nevertheless it appears certain that they at times eat each other. A few times their remains have been found in the snares. A few remains were observed in the glass cases in which they were sometimes kept in the laboratory. Quite a number of times remains were found in the collecting vials the next morning when three or four were left in the same vial over night.

When bits of dirt were thrown into the snares, the spiders ran away quickly. Blind beetles were caught and tossed into the snares. At the instant when the beetles struck the snare, the spiders ran and with a jump seized the victim. Neither the web, nor spider, nor both together were strong enough to hold the beetle. Large flies were also thrown into the snare with the same results. The spiders always seized their prey and held on tenaciously until the last second. Small flies and mosquitoes were likewise tossed into the snares. In each case the spider made a quick run and with a jump seized the victims and held

[1]Here as elsewhere used in broad sense to include both *Thysanura* and *Collembola*.

on so firmly that both prey and spider were torn loose from the web by picking the prey up with forceps.

Not one specimen has even been seen drinking water. Since they always live in a saturated atmosphere all the water required is probably absorbed through the skin.

In captivity I have fed *Meta* flies, mosquitoes, gnats and various species of other arachnids smaller than themselves. When two or more of them are placed in the same case the largest invariably devours all the others in a short time.

In the caves *Metas* have been observed at various localities eating gnats, mosquitoes, flies and cave crickets. One was seen eating a small moth and another an old dried myriopod.

Enemies.

As far as my observations go, these spiders have no enemies besides themselves. It is very seldom that one can find the remains of a specimen in the webs and as mentioned above no case of fighting has ever been witnessed. At "63" almost a mile from the entrance the only other insect found was the blind beetle. Doubtless carefully repeated observations at this locality will prove that other insects are also present.

Temperature.

During the last three years a series of temperature records[1] have been taken at "10," or in the "Big Room." For 1907 the temperature is as follows: 11.5° C. for January, February, March, April and May; June 11.7°; July 11.9°; August and September 12.7°; October 12.2°; November 11.9°; and December 11.7°. Since food is more abundant and the three necessary physical conditions are evidently suitable at "2," "9" and "38," we can probably attribute the small number of specimens to the uneven temperature. Again, since food is extremely scarce a half mile from the entrance while the number of specimens is few, perhaps the small number of spiders is due to the scarcity of food. Midway between these two localities food is comparatively plentiful, the temperature is practically even throughout the year, and this combination is probably responsible for the great number of individuals.

[1]Eigenmann, '09.

Specimens have been kept in captivity in the laboratory throughout the year. In cold weather they are less active than in warm and are very fretful when the vials become too warm, and often die.

Courtship.[1]

November 16 two were seen copulating. In order to see them I got too close and my breath irritated the web. This caused them to separate. After an absence of five minutes I returned and found them together again. A second time they were disturbed. Returning after an absence of fifteen minutes, they were found close together. While copulating, they were lying one under the other with anterior and posterior ends reversed and with the ventral parts of their cephalothorax in contact. November 19, a pair was seen pairing. December 17, two were observed copulating, these were both under the snare, and the anterior and posterior ends of the cephalothorax were reversed. The dorsal surface of the cephalothorax of the male was pressed against the corresponding ventral part of the female. The male placed his palp on the epigynum once, this lasted only a few seconds, then they parted. December 14 and 22, two couples were seen copulating. Those on the latter date were first seen at 2:25 P.M. and then disturbed by breath. At 2:27 they were together again after the male had circled once around the female. This pair was on top of the horizontal snare all the time. The male used his palps alternatively three times in three minutes, each time lasted only a few seconds. They were disturbed at 2:30. January 4, two were observed copulating June 7, a male was seen courting a female. Both were under the snare within one and one-half inches of each other. They were first seen at 3:45 P.M. When the male tried to advance toward the female, she caused him to keep his distance, the result of which caused the male to circle completely around her clockwise in six minutes. Most of the time she kept the posterior end of her abdomen toward him, while he had his head facing her all the time. At 4:00 I left them and a half hour later upon my return they were still in the same position. They were placed in a vial, taken to the house and were put in a case. In this

[1]McCook, '93, describes only courtship of outside forms.

case they lived two weeks where they died. This pair like all others when in captivity had no inclination to mate.

Cocoons and Eggs.[1]

One cocoon was made in a glass case October 4. It contained six eggs. Another was made in a case December 11. January 21, one with five eggs was constructed in a vial. Other cocoons were made in vials on the following dates: April 28; May 5, 8, 22, 27 and 31; June 3, 25 and 28; July 8 and 27; August 6, 19 and 21.

On the following dates cocoons with eggs were collected in the caves: October 8, one with two eggs; one with eight eggs, January 20; January 26, one containing eight newly hatched spiders and one with seven eggs; another with seven eggs March 2; March 16, two cocoons, one with eggs and the other with newly hatched; May 24, one with four eggs; July 2, one with four eggs; July 19, at various localities collected seven cocoons, one of which contained eight eggs, and two others each held four young, at various localities in Upper Dalton August 22 collected seven cocoons. Some of these contained young just hatched, and others young ready to leave the cocoons.

The cocoons in the caves are usually constructed in secluded places and are difficult to find unless one examines every little crevice and looks under the ledges of rocks very carefully. Sometimes they are found attached to the underside of rocks lying on the floor, but more often under little ledges of rocks and in the acute angles of small crevices.

In color they are snow white and are disc-like in shape. The flat part of the disc is fastened firmly to the rocks. The average size is 6 mm. in diameter by 3 mm. in depth, although sometimes a cocoon containing the minimum number of eggs is as large as one containing the maximum number. I have never been fortunate enough to witness a female making her cocoon, but on examination, a coocon is composed of a more or less firm and closely woven circular base. The eggs are piled into a heap in the middle of this base and then the convex part is spun over them in such a crude and unsubstantial way that one can generally count the eggs through this covering. In detaching the

[1]Montgomery, '06, describes the cocoons and eggs of an allied outside form.

cocoon from the rock one must use precaution for fear the eggs fall through the covering.

In number the eggs vary from two to eight with five for an average cocoon. They are transparent whitish in color and are perfect spheres with an average diameter of 0.6 mm. During the embryological stages, they soon take on a yellowish color, become oblong in shape, and the outline of the embryos is discernible through the covering. Some of these embryos assume the shape of the profile of a man's head.

Young.

When hatched they remain for an indefinite period inside the cocoon and when strong enough emerge through a small circular hole.

March 22, three of the seven eggs in a cocoon collected March 2, hatched; April 3, two of these spiderlings were dead, they with the remaining eggs were covered with mold. June 4, two of the four eggs in a cocoon collected May 24, were hatched, one spiderling was dead and the other alive on this date. Neither one had any eyes. July 29, a cocoon collected July 19, was examined and contained three young. Each one was examined both alive and dead. All eyes, except the anterior middle ones, were discernible. Female no. 139 made a cocoon and laid four eggs May 5. On May 23 all four eggs were hatched, but the young were still inside the cocoon. Each spiderling had all eight eyes except the anterior middle ones. The eyes had a uniform diameter of 0.018 mm. While alive under the microscope their little eyes shone like small electric lights. Their mother had no signs of external eyes. Many other newly hatched spiderlings were observed both alive and dead. The anterior middle eyes are never discernible. In some, the other six eyes are present and in others no eyes can be seen. All the other eggs laid in the laboratory failed to hatch. Perhaps this was due to uneven temperature.

The young are much thicker-set than the old. The legs are thick and stubby. The cephalothorax and legs are transparent whitish while the abdomen is light cream in color. The latter has a few longitudinal rows of hairs. The length varies from 0.6 to 0.8 mm.

Moulting.

While in captivity seven individuals moulted, three of which were found dead shortly after the skins were cast off. The deaths were probably partially due to an excess of water in the vials for immediately after the old skins were shed the spiders lay lifeless in the water. The skins were suspended by threads to the upper side of the vials. The moults show that the skin splits on each side of the cephalothorax at the dorsal side of where the legs are attached. Hence, the moulted skin of legs and mouth parts adhere to the ventral half of the moult, and the corneal cuticle belongs to the dorsal half. All the old hairs are shed along with the skin, new ones take the place of the old which causes the color to be perceptibly brighter. The moulted skins of the abdomen were either missing or rolled up into little wads so that one could not tell precisely how they were cast off.

Mortality.

In the caves one never finds dead specimens. In captivity mortality is not great. The most important requirement is to place them in a saturated atmosphere as soon as caught. Two thirds of the specimens were found dead in the collecting vials the following morning when left in the vials without a drop of water over night. It is impossible to keep them long in anything not air-tight, however careful one is to keep them supplied with water. The best device is small vials with air-tight cork stoppers. In such vials they may be kept for months without food. One caught September 10 was placed in a small vial containing two drops of water. On January 20 more water was added which almost drowned the specimen. On January 26 it died. During all this time it had had nothing to eat. Another individual was placed in a small vial January 7, and died April 19, due to lack of moisture or food.

In captivity they quite often die soon after moulting. In vials sunshine kills them in a few minutes. The heat from a student's lamp is also fatal.

Light Experiments.

In the caves one may throw the light from a carbide bicycle lamp on *Troglohyphantes* for a half hour or more without pro-

ducing any effect. Such is not true with *Meta*. Just as soon as the light strikes their eyes, they run into the dark. If the light is repeatedly thrown on their eyes, they may be turned in any direction and often can be driven into places where the light cannot reach them.

The following apparatus was used in the laboratory: For the adult *Metas*, slender 10-inch bottles; for the medium sized *Metas*, 6-inch test-tubes; for the spiderlings of *Meta* and for *Troglohyphantes*, small 5-inch vials. The closed end and the lower half of each vessel were covered with black carbon paper. The open ends were securely closed with air-tight cork stoppers. One specimen with one or two drops of water were placed in each vessel. In a very short time the water forms a thin film of moisture all over the inner surface of the vessel. This saturated the air in both ends equally. The vessels were then placed on an inclined rack by a south window in order to give each an equal amount of light. Occasionally they were rotated so that the light always fell directly upon the spiders' eyes. At various times the carbon paper was transferred to the cork end, thus throwing the specimen into the light or dark as the case may be. Those that were strongly negatively phototropic never lost much time in finding the dark end, regardless of the number of times the carbon paper was changed. Such individuals often pass into the dark in three minutes. The few that were strongly positively phototropic always changed from the dark end to the light end whenever they were thrown into the dark. Some were thus experimented with for thirty days, but experience taught that their actions were reliable for only the first four or five days. Darkness and cloudy weather had much to do with the final results. Time was counted from the period when first placed in the vessel, and each morning when first observed until 6:00 P.M. each day for four consecutive days. When first placed in the vessels and for a short time after the carbon paper was transferred, they were noticed every few minutes, after that irregularly five times every day.

The adult *Metas* were always in the dark end during clear and cloudy weather, and always in the light end when it was dark. The medium sized *Metas* were always found in the dark

end in clear weather, one half the time in the dark end during cloudy weather, and usually in the light end when dark. The spiderlings of *Meta* remained in the dark end one half the time in clear weather, one third the time in the dark end during cloudy weather and most of the time in the light end when dark.

All eight eyes in the *Metas* were present and presumably well developed. The anterior middle ones were generally a little smaller than the others and occasionally an eye was found among the others which was about one half size. In all these experiments forty *Metas* were used.

The following table gives the results of the light experiments for *Troglohyphantes*. On the left is entered the number of the specimen, the locality, the sex, age (whether mature or immature) and the four sets of eyes, AME stands for anterior middle eyes, PME for posterior middle eyes, ASE for anterior side eyes, and PSE for posterior side eyes. All measurements were made with a micrometer slide inside the two inch ocular with two thirds or low objective. When the eyes were scarcely discernible the one-inch ocular was substituted for the two-inch. As a unit of measurement for the eyes one fifty-fifth of a millimeter (0.018 mm.) is employed. The fractional parts of this unit are only approximate. The +'s are used when the eyes are joined together, if separate —'s are employed. P stands for pigment speck. The remainder is self-explanatory.

The thirty examples included in the table were selected, not on a phototropic basis, but to represent the various localities, the age, size, degeneration of the eyes, and coloration. If another table were made from the specimens not included in this one, the results would be similar. If a correction could be made for cloudy weather and for the time occupied in going into the dark, the total per cent. of 49 for those in the dark column would be considerably larger than the total per cent. of 51 for those in the light column. In all these experiments 225 specimens have been used and I am positively certain that the results as given in the table are correct.

Summarizing the following table and other data not included therein we have the following statements:

Twenty-six per cent. of all the individuals examined had no

LIGHT EXPERIMENTS FOR TROGLOHYPHANTES CAVERNICOLA KEY.

No.	Loc.	Sex.	Age.	Size.	AME.	PME.	ASE.	PSE.	Color Abd.	Color Ceph.	Light, Hrs. and Min.	Dark, Hrs. and Min.	Light, Per Cent.	Dark, Per Cent.
397	9	♂	Im.	2.2	.5+.5	.7—.7	.7— .7	.7— .7	light	pink	11.40	24.45	38	62
712	9	♀	Mat.	2.8	.5—.5	1—.5	1— 1	1.5—1.5	brown	"	15.20	21.05	42	58
322	10	?	Im.	1.0	0— 0	0— 0	0— 0	0— 0	light	light	10.00	25.15	28	72
324	10	♂	"	2.5	.5+.5	1— 1	0— 0	0— 0	"	pink	15.15	20.00	43	57
327	10	♀	Mat.	2.6	0— 0	0— 0	0— 0	0— 0	brown	"	13.00	22.15	37	63
714	10	♀	Im.	2.7	.5+.5	1— 1	1— 1	1— 1	"	light	8.25	28.00	23	77
325	13	♀	Mat.	2.8	1— 1	2— 2	1.5—1.5	1— 1	"	pink	30.15	5.00	85	15
326	13	♀	"	2.5	1+ 1	2— 2	1— 1	1— 1	light	"	18.00	17.15	51	49
399	13	♀	"	2.8	.5+.5	.5—.7	1— 1	1— 1	"	"	17.55	18.30	45	55
705	14	♂	"	2.7	.7+.7	1— 1	1— 1	1— 1	"	"	23.10	13.15	63	37
747	14	♀	Im.	2.0	0— 0	2— 2	2— 2	2— 2	"	"	2.45	33.35	8	92
729	14	♂	Mat.	2.8	.7—.7	1— 1	1— 1	1.5—1.5	"	"	22.05	14.15	60	40
741	14	♀	"	3.0	0— 0	0— 1	.7— 1	0— 1	"	"	15.50	20.30	44	56
709	19	♀	"	3.0	0— 0	0— 0	0— 0	0— 0	"	"	16.05	20.20	45	55
731	19	♀	"	3.0	.7— 1	1— 0	1— 1	1.5—1.5	brown	"	30.30	5.50	83	17
735	19	♀	"	3.1	1— 1	1— 1	0— 1	0—1.5	dark	"	30.50	2.30	91	9
736	19	♀	"	3.0	.7— 1	1— 1	0— 1	1.5— 2	light	"	36.20	0.00	100	00
359	38	♀	"	2.8	.5—.5	P— 1	0— 0	0— 1	brown	"	13.05	21.40	38	62
374	38	♀	"	2.9	.5—.5	.7—.7	.7— 0	.7— .7	"	"	29.30	9.00	76	24
356	38	♀	?	?	.5—.5	0—.7	0— 0	0— 0	?	"	28.05	6.40	81	19
355	40	♀	Mat.	2.8	.5— 1	.5—.5	1— 1	1— 0	brown	"	15.10	19.35	43	57
371	40	♀	"	3.0	0— 0	0— 0	0— 0	0— 0	light	"	2.25	32.20	7	93
375	43	♀	Im.	3.0	0— 0	0— 0	0— 0	0— 0	dark	"	16.30	22.00	43	57
753	43	♀	Mat.	3.2	.5— 1	1— 1	.7— .7	0— 0	"	"	33.40	0.00	100	00
755	43	♀	"	2.4	1— 1	1— 1	0— 0	1— 1	"	light	25.45	7.55	76	24
725	46	♀	"	3.0	.7—.7	1— 1	.7— 0	1— 1	brown	pink	12.40	23.00	35	65
726	46	♀	"	2.9	0— 0	1— 1	1— 0	1— 1	black	"	10.25	25.15	29	71
721	50	♀	"	3.2	.7—.7	1— 1	1— 1	1— 1	dark	"	14.55	21.20	41	59
722	50	♂	Im.	2.3	0— 0	0— 0	0— 0	0— 0	light	light	12.05	24.10	33	67
331	57	♂	"	2.0	0— 0	0— 1	0— 0	0— 0	black	pink	17.45	17.15	51	49
											Total 549.25	522.30	51	49

[? Abdomen was lost.]

external eyes. Sometimes the eyes are not in their natural position. Often black pigment specks are found where the eyes are absent. The largest eyes are two fifty-fifths millimeter (0.036 mm.) in diameter, being twice as large as those of the newly hatched, but such individuals are comparatively rare. Hence as a rule, the eyes do not grow larger after birth, while the specimens more than thribble themselves in size. Neither locality nor size of the specimen determines the degree of degeneration in the eyes, or the shade of coloration. Generally, the lighter colored the individual, the more degenerated the eyes, and vice versa. Specimens totally devoid of eyes always stay in the dark more than fifty per cent. of the time; those with one or more

eyes may stay either in the light or dark more than fifty per cent. of the time, the per cent. depending on the amount of degeneration.

Humidity.

Apparatus.—Glass tubes with one-half-inch bore and twelve inches long were used. The opening and three inches of one end were covered with black carbon paper. The other end was closed with a cotton cloth. A spider and two drops of water were placed in the light end of each tube and the tubes were placed in the light the same as in the light experiments. When first placed in the tubes the specimens wandered from one end to the other. In just a few moments they ceased their wandering and remained within reach of the drops of water. As it was impossible to watch these experiments all the time, quite frequently the drops of water evaporated before new ones could be added. Sometimes when the tubes became dry, the spiders were found in the dark end, other times in the light end. Under such conditions some were able to live only one or two days, some four or five days while others survived ten days. Out of two dozen individuals used not one at any time was ever found in the dark end when the light end was wet. Each specimen was examined, some had eyes and others were devoid of eyes. Judging from the preceding experiments on light the specimens devoid of eyes should have been found in the dark end at times, providing there was no other factor stronger than negative phototropism. Since these specimens remained near the drops of water all the time instead of going into the dark, we conclude that humidity is a stronger factor than negative phototropism.

The same experiments were prosecuted with the spiderlings and medium sized specimens of *Meta*. At times these were found in the dark end when the light end was wet, therefore probably humidity with them is not greater than negative phototropism.

Change of Humidity.

Apparatus.—The same tubes as used in the preceding experiments for humidity, also a hygrometer was employed. A specimen was placed in each tube and was observed several times each day. The following results show the relative humidity and the number of hours and minutes various individuals lived.

No. of Specimens.	Relative Humidity.	Hrs.	Min.
4	38—36	3	15
2	70—66—59	5	30
1	38—36—44	8	15
4	63—93	8	15
1	95—82—84—88— 92—78	23	35
1	81—60—76—73—100—75—89	31	45
1	100—65—55—60— 72—66—60	33	45

On various dates at the entrance and at the different localities in the caves the relative humidity was recorded. At the entrance it varied considerably on different days, but in the caves, the hygrometer always stood at 100 (saturation point).

These arachnids always live in a saturated atmosphere and it is impossible for them to survive long outside the caves where the variation in the degree of humidity is great. As a general rule the higher the relative humidity (with but a gradual and small amount of variability), the longer they live. Since the above experiments were prosecuted from May 18 to June 8, when the change in temperature was not such as to materially affect these spiders, we must attribute their deaths to the hygrometric conditions.

Summary.

1. *Troglohyphantes cavernicola* Keys. is found everywhere in these caves, where the three following necessary conditions exist—total darkness, a saturated atmosphere, and a suitable place for the construction of snares.

2. The first pair of legs are used as tactile organs.

3. All small winged insects, thysanurans and small myriopods serve as food. Scarcity of food does not entirely limit their distribution.

4. They have no known enemies other than themselves.

5. While temperature outside the caves does not materially affect the adult spiders themselves, it is probable that to the even temperature at localities between 600 and 1,700 feet from the entrance is due the great number of specimens found at this place.

6. Courtship is similar to that of some outside forms.

7. Cocooning is rudimentary. The eggs are few and comparatively large.

8. The young are white and are thicker set than the adults. Some are hatched with eyes, while others are entirely blind.

9. Moulting is comparatively rare and is often fatal. There are all shades from white to black in coloration.

10. In size the eyes vary from a small pigment speck to 0.036 millimeter in diameter. As a rule, after birth the eyes cease to grow while the specimens more than thribble themselves in size. Twenty-six per cent. of all the individuals are entirely devoid of eyes.

11. The degree of degeneration in the eyes and the shade of coloration are not determined by either locality or size of the specimen.

12. The lighter colored the specimen the more degenerated the eyes.

13. The more degenerated the eyes the greater the negative phototropism, and vice versa.

14. Humidity is a stronger factor than negative photropism in determining the location of specimens in the experimental tubes.

15 Change of relative humidity is fatal in a few hours

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EXPLANATION OF MAP.

Shawnee Cave (the outlet). Sec. I., No. 1.
Closed chamber caused by collapse of roof at Sec. I., Nos. 2–3.
Cascade. Sec. I., No. 6.
Double passage. Sec. I., Nos. 7–8.
Old cross cave. Sec. I., Nos. 9–10.
New passages. Sec. I., Nos. 1–8 and 11–13.
Opening in roof leading to *upper older* levels of cave. Sec. I., No. 14.
"Big Room." Sec. I., Nos. 15, 16, 17, 18, 19, 20, 21, 22.
"Fallen Rock." Sec. I., No. 31.
Lower Twin Cave. Sec. I., No. 32.
Upper Twin Cave. Sec. I., No. 33.
Roof too low for passage of boat. Sec. I., No. 34.
Deepest water in cave, 10 feet 4 inches. Sec. I., No. 35.
Lower Dalton Cave. Sec. I., No. 36.
Upper Dalton Cave. Sec. I., No. 37.

FIG. 1. Map of Shawnee Cave, section 1, from Shawnee to Lower Dalton. Length 4,453 feet. Scale 200 feet to the inch.

Explanation of Map.

Upper Dalton Cave. Sec. II., No. 37.
"Cross bedding" in limestone. Sec. II., Nos. 46–47.
"Old passages." Sec. II., Nos. 56–57.
Obstruction past which boat cannot be taken. Sec. II., No. 63.
End of exploration. Sec. II., No. 64.

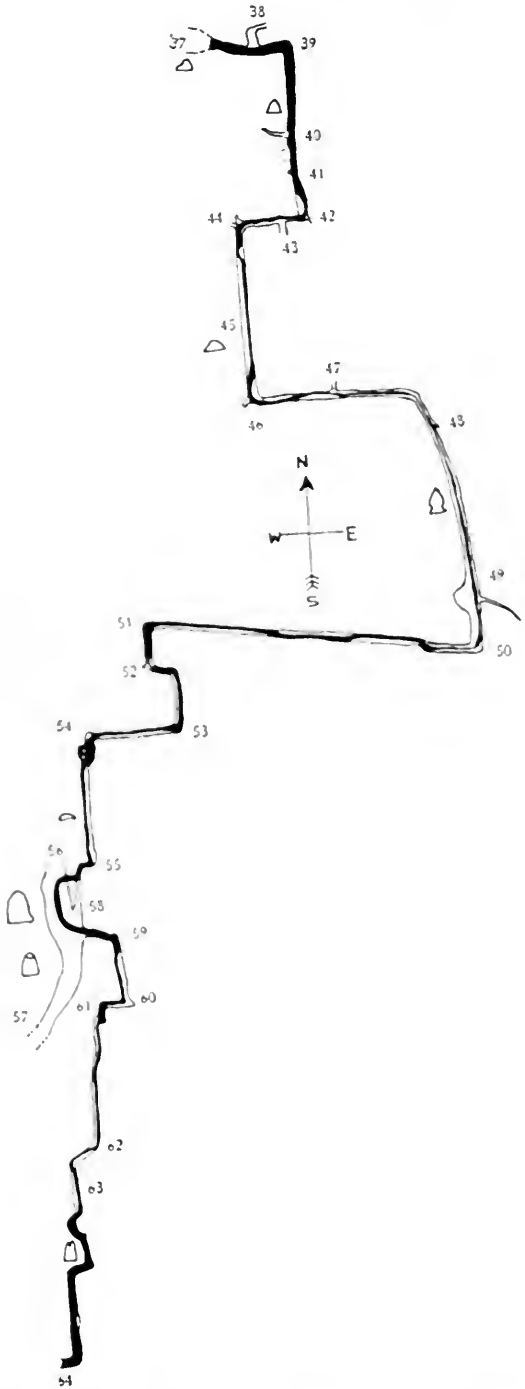


FIG. 2. Map of Shawnee Cave, section 2, from Lower Dalton to unexplored

A NEW SPECIES OF PARAMECIUM (P. MULTIMICRONUCLEATA) EXPERIMENTALLY DETERMINED.[1]

J. H. POWERS AND CLAUDE MITCHELL.

On September 27, 1909, I received from Dr. Powers two sample cultures of Paramecia with the request that I investigate them as to type and purity of culture. To this end I first killed, fixed, mounted and examined 1,000 individuals. They proved to be neither typical *Paramecium caudatum* nor *Paramecium aurelia*, although most of their characters differed but little from these well-known types. Their length ranged between 144 and 288 μ. Their anterior end was a little blunter and the posterior end a little more pointed than even in *P. caudatum*. The cytoplasm was more dense and more opaque. Their chief difference, however, from hitherto described types of Paramecia lay in the matter of the micronucleus, for, instead of the single micronucleus of *P. caudatum* or the two micronuclei of its variety *P. aurelia*, there is a number of very small bodies, evidently micronuclei, ranging in diameter from about .7 to 1.15 μ (Fig. 3). The characteristic position of micronuclei is fully retained, these bodies lying either in slight grooves or in shallow impocketings of the macronucleus. Like the micronuclei of other types, these delicate bodies are always surrounded by a nuclear membrane.

Of the 1,000 individuals examined 875 distinctly showed from two to six of these small micronuclei, 124 showed apparently no micronuclei whatever, while one appeared at first to possess a micronucleus of the type found in *P. caudatum*. This single instance, however, turned out upon careful study to be a case in which a detached fragment of the macronucleus chanced to simulate in size and appearance the regular micronucleus. As to the 124 which appeared without micronuclei, entire degeneration of these bodies may have been possible, but it is more probable that a slight overstain obscured them, especially when lying behind the macronucleus; the same explanation is doubt-

[1] Studies from the Zoological Laboratory, the University of Nebraska, No. 101.

less true of the individuals in which but two or three micronuclei were found, others existing but in a less visible location.

Finding thus that the cultures in hand contained nothing but this same type of *Paramecium*, I next proceeded to test the permanence of the type. On October 9, I isolated five individuals, placing each in a clean watch glass containing a definite proportion of sterile and bacterially infected water. These individuals, however, lived but a day. I then again selected five more, varying the proportions of the fluid media. Of these, the two which were placed in water containing the highest percentage of bacteria lived, while the others did not. Three more were started in the same manner as the two successful ones and all these gave living cultures. Of the five living cultures thus obtained two proved much stronger than the others, despite the fact that the conditions were kept as constant as possible in all cases; these two increased rapidly in numbers, while the others increased but little and finally died out after five weeks.

From these two strong cultures, forty individuals were killed, stained and mounted on November 22 and about fifty more on December 17. All of these proved identical in type with the original wild stock. The minute micronuclei were present as before, and again seemed to vary from three to seven in number, which difference depended, in part at least, upon the stain and the transparency of the individual.

Unfortunately during Christmas week extreme cold weather and partial failure of heating plant caused the death of all isolation cultures, the original culture, however, remaining. From this latter, single individuals were again isolated and new cultures started on January 31, the culture media being varied as in previous instances. This resulted again in cultures of varying degrees of strength. One of the best, which I will designate as culture X, was chosen and tested by mounting a number of individuals all of which again proved to be of the multimicronucleate type. This culture X was now accordingly taken as a basis for all further work. From it six cultures were started, the medium being modified in this case by the use of different proportions of agar agar infected with the customary bacteria.

Of these six cultures one, culture Y, was worthy of especial

note in that it produced a few conjugants. Early in April this culture became infected with a minute unicellular alga and, possibly as the result of this, the paramecia became more active and increased more rapidly in number. They also ingested the algæ until they became greenish in color. On April 15 six pairs of conjugants appeared. Three of these were killed in about the three-hour stage of conjugation, another in about the seventeenth hour of conjugation, while the other two pairs were isolated, allowed to complete the act of conjugation, and the ex-conjugants used to start new cultures. It was hoped that stronger cultures would thereby be obtained, but this did not follow. They lived and divided slowly for about three weeks only.

The pairs of conjugants which had been killed were stained and mounted in toto, and are of interest as showing, not only that this type of *Paramecium* is capable of conjugation, but something of the nuclear phenomena undergone during the process. In all cases the micronuclei, or at least a part of them, could be made out. In those killed at the three-hour stage (Fig. 1) all were in pairs, indicating no doubt the customary divisions preceding nuclear exchange. In one case three of these pairs were really single nuclei in advanced division. With difficulty the nuclear membrane could be made out, extending, as in the case of the larger dividing micronuclei of *P. caudatum*, between the separating portions of the dividing nucleus. The micronuclei forming the pairs in these three-hour conjugants were smaller than those in non-conjugants. The macronucleus in this stage is still unchanged except that its surface is more or less furrowed.

In the pair of conjugants killed at the seventeen-hour stage the micronuclei are also present, some again in pairs or in division, some single. The macronucleus on the other hand has now broken up into bands and curved segments, simulating a reticulum. This breaking up of the macronucleus at an early stage does not occur with *P. caudatum*, and, in case further study shows it to be habitual with the present type, this will constitute further proof of its independence.

The limited number of conjugants at our disposal and the consequent inability to procure all the stages have prevented

our demonstration of the actual nuclear exchange during conjugation, but such exchange is naturally to be inferred from the preparatory division of the micronuclei and from subsequent breaking down of the macronucleus. Every feature of the few conjugating pairs examined indicated normal processes and conditions.

Although the cultures started from the ex-conjugants fared so poorly it is worth noting that shortly after the latter were discovered in culture Y this culture underwent a rapid acceleration in growth and division. This may have been due to undetected cases of conjugation occurring in the culture, or on the other hand it may have been the result of some external stimulus, which itself had caused the conjugation. From this rapidly growing culture two further lots of Paramecia have been mounted, and, as before, all prove to be of the multimacronucleate type. Sections have also been made and stained with iron hæmatoxylin (Fig. 3) from different groups mounted during the year. They have fully borne out the results of the more numerous toto mounts.

All told the cultures have been conducted, the first group for three, and the second for five months. They have not been as strong as could be wished, but the best cultures obtained showed at least no tendency to die out and at the close of the work culture Y was multiplying more rapidly than at any previous time. Had our temperature conditions been more uniform and favorable, we should probably have been able to rear much more copious cultures. The entire uniformity of the type throughout these cultures seems good evidence for its permanence and the probability that it deserves specific rank.

CLAUDE WM. MITCHELL.

I can fully vouch for the methods and results which my student, Mr. Mitchell, has recorded in the first part of this paper. I may speak a few words further as to my own experience with *P. multimicronucleata*. It is not, in the writer's vicinity, a rare or accidental type. Throughout a number of years of work in eastern Nebraska it has been a frequent and troublesome intruder in my *Paramecium* cultures. The most persistent efforts

have often failed to procure, from wild stock, pure cultures of *P. caudatum*. A portion, usually the bulk, and frequently the whole, of any culture obtained from pond or river water would turn out to be of this multimicronucleate type.

I did not at first recognize the minute micronuclei. I regarded the individuals, which careful and elaborate technique showed to be lacking in the typical micronuclei of *P. caudatum* and *P. aurelia*, as degenerates in the sense of Maupas' contention. As however the hypothesis of the degeneration of the micronucleus became more and more discredited, I reëxamined mounted slides of these Paramecia under high magnifications, with the result that the minute bodies in question were visible in every case. That this type of *Paramecium* was not related to degeneration was further shown by the fact that many pure cultures, unlike those with which Mr. Mitchell has labored so assiduously, have been vigorous and strong growers. I may further mention the fact that in several very large aquaria supplied with running water and a small amount of fresh meat added occasionally, this type of *Paramecium* apparently maintained itself continuously for several years. As often as the organic matter was supplied the animals would multiply and appear in vast swarms in the corners and protected portions of their space; whenever examined they proved of this type and of this type only.

The existence of an undescribed species of *Paramecium* seems improbable. The protozoa are considered of universal distribution, and *Paramecium* is the most-studied genus in existence. Nevertheless much of the study of mircoörganisms is superficial; many have failed to develop a suitable technique, easy as this is, for the certain demonstration of micronuclei; and as to the hypothesis of universal distribution, it is certainly assumed much further than it is proven. Thus, for the last six years, I have made careful search among cultures derived from very numerous wild stocks, for *Paramecium* of the *aurelia* type, *i. e.*, with the well-known two micronuclei. But, aside from a very few isolated individuals derived experimentally from *P. caudatum*, not a single example has been found.

All in all, it seems that, in the light of Mr. Mitchell's results, the type in question deserves specific rank, although this rank

rests upon nuclear differences only. The external characters mentioned by Mr. Mitchell, although holding good for the material investigated by him and for many other lots as well, are not universal, nor have I noted any external character that is. When *P. multimicronucleata* is grown in the same culture with any given strain of *P. caudatum*, the two can usually be readily separated by one or more external characters. Frequently the *P. multimicronucleata* are uniformly larger than the accompanying *P. caudatum*, but they do not exceed the known dimensions of the commoner form, and, in some cultures they are uniformly smaller. So with length of groove, form of ends, opaqueness, etc. The most uniform character that I have seen is that the new type is a little more cylindric in form, bulging less at the point of greatest diameter; but *P. caudatum* approaches this form in starved cultures.

The propriety of basing a species upon nuclear characters only depends of course upon their constancy. Calkins has shown that the types of *Paramecium* with single and double micronuclei respectively are not wholly constant, occasional transitions taking place in both directions. He, therefore, pronounced the types varieties. But the conclusion has again been called in question, the infrequency of the transition leading Kofoid to re-interpret the phenomena as instances of mutation.

I have myself been conducting, during a considerable part of the present year, preliminary experiments on *P. caudatum*, subjecting them to different conditions with a view to ascertaining their possibilities of variation. The only striking results have occurred as the consequences of great changes in feeding habit.

P. caudatum is almost exclusively a bacteria feeder. But as Mr. Mitchell has recorded they occasionally deviate to other minute vegetable organisms. This year I have succeeded in inducing a certain percentage of the individuals from a pure culture of very large and strong growing *P. caudatum* to feed on minor animal organisms, first on flagellates (*Chilomonas*) and then, to a considerable extent, upon smaller ciliates. These very striking changes in food habit produced very striking variations in the Paramecia, both nuclear and cytoplasmic. I will not

describe these at the present time save in so far as they relate to the present discussion. Many of the nuclear changes were erratic and possibly pathological: Macronuclei greatly enlarged, micronuclei unchanged, or sometimes apparently absent, or again enlarged, even more in proportion than the macronucleus, and sometimes divided.

Among the large mass of such material, stained, mounted and examined, I discovered a very few instances of individuals with two typical micronuclei. In fission these micronuclei divided simultaneously and normally. The number of these individuals was very few, probably not exceeding one to several thousand, but they confirm, to some extent, Calkins's observation that *P. aurelia* may arise from *P. caudatum*.

Among the different types of variants I sought assiduously for examples of *P. multimicronucleata*. But none of the exact type were found. Evidently this type is farther separated from *P. caudatum* than is *P. aurelia*. A considerable number of individuals were found however which showed an approach to *P. multimicronucleata*, in that the micronucleus was divided, usually, again, into but two bodies, perfectly normal in appearance, but much smaller than the typical micronuclei of the genus, though a little larger than those of the new type. This variant was one of the most constant and frequent results of the changed diet. In other characters, however, it did resemble closely *P. multimicronucleata* or, for that matter, any recognized type of the genus. I regard it merely as an instance of the well-known law that a powerful stimulus to variation applied to any species brings out, not only new characters, but characters of existing allied species as well. The phenomena, to the writer, serve to confirm, rather than to refute, the specific independence of the new type. But they are of interest in themselves as showing possible lines of experiment leading to nuclear variation. In the present instance it seemed especially worth while to record them, and indeed this is the chief reason for the entire study, in that *Paramecium* is more and more being made the subject of extensive experimental research. So far, little of this study has had regard to other than external characters, but this admitted limitation must soon be remedied, and to this end it is essential that we know the types

of nuclear structure present in the different species or varieties of the genus as well as the lines of variation to which they are subject.

J. H. POWERS.

UNIVERSITY OF NEBRASKA,
July 30, 1910.

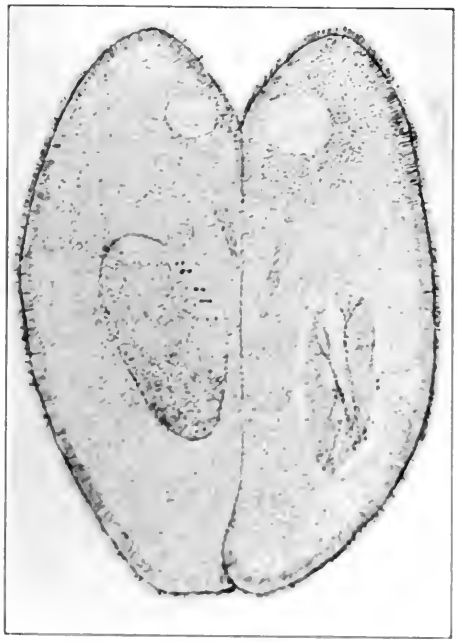
Explanation of Plate I.

Paramecium multimicronucleata.

Fig. 1. Conjugation near the three-hour stage, showing micronuclei in pairs or in division.

Fig. 2. Conjugation at about seventeen hours. Macronucleus already broken into band-like portions. Micronuclei visible in part, in pairs or single.

Fig. 3. Section of typical *P. multimicronucleata*, an unusual number of the micronuclei chancing to lie in one plane.



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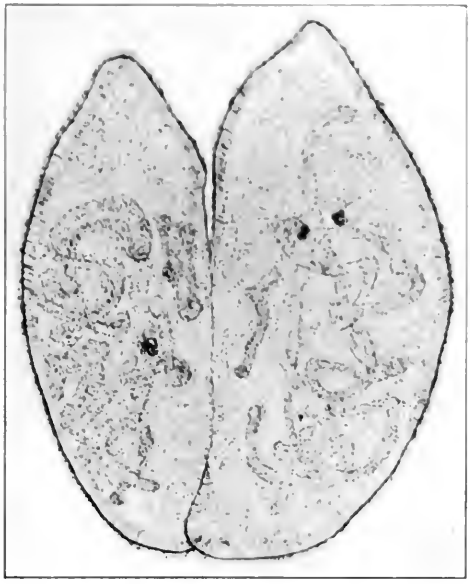


2

THE CENTRAL NERVOUS SYSTEM AS A FACTOR IN THE REGENERATION OF POLYCLAD TURBELLARIA.

C. M. CHILD.

Some years ago L. V. Morgan[1] described a series of experiments concerning the effect of removal of the cephalic ganglia upon the course of regeneration, particularly of the anterior region, in the Californian polyclad, *Leptoplana littoralis*. Her conclusion is that under certain conditions, *i. e.*, particularly in the presence of tissue anterior to the ganglia, regeneration occurs as readily and as completely in the absence of the ganglia as when they are present. At about the same time the results of my own experiments on *Leptoplana tremellaris* appeared.[2] I found that when half or less than half of the ganglionic tissue was removed, regeneration might be as complete and as rapid as when the ganglia were uninjured, but that when more than half of the ganglia was removed, regeneration was not only slower but less complete than when they were uninjured.

I also found, however, that groups of eye spots not infrequently appeared in cases where the ganglia themselves were, so far as could be determined, completely removed, and that the amount of anterior new tissue in all such cases was greater than in those cases where eye spots did not appear, though regeneration was never complete.[3] My general conclusion from these experiments was that the central nervous system in these forms acted as a functional stimulus to the growth of new tissue, increasing both the rapidity and the amount of growth.

The above mentioned experiments of L. V. Morgan seem to

[1]L. V. Morgan, "Incomplete Anterior Regeneration in the Absence of the Brain in *Leptoplana littoralis*," BIOL. BULL., IX., 3, 1905.

[2]Child, "Studies on Regulation, "V.—The Relation between the Central Nervous System and Regeneration in *Leptoplana*: Posterior Regeneration," *Journ. Exp. Zoöl.*, I., 3, 1904. "Studies on Regulation, VI.—The Relation, etc.: Anterior and Lateral Regeneration," *Journ. Exp. Zoöl.*, I., 4, 1904.

[3]Child, "Studies on Regulation, VI.," Figs. 15–19, p. 522, Figs. 41–43, p. 526, also p. 529.

indicate that under certain conditions this is not the case. In view of the apparent disagreement between her results and my own further experiment seemed desirable and during the autumn of 1907 and the summer of 1910 I took the opportunity to examine several species of Leptoplanidæ which occur at La Jolla, Calif., with reference to this point. In 1905 I had worked with *L. littoralis* at Pacific Grove and obtained results similar to those described for *L. tremellaris*.

My conclusions from this later work are essentially the same as those reached in my earlier paper. Removal of the ganglia with as little of the surrounding tissue as possible always results in decreased rapidity and completeness of regeneration, whatever the method of operation employed. In many cases, however, groups of eye spots appear in the new tissue, even when the ganglia are wholly absent, and in such cases the regeneration is always more rapid and more nearly complete than when the eye spots do not appear.

When the ganglia are removed by a cut from one side of the head, as in some of Morgan's experiments, more new tissue is often formed, or it forms more rapidly, in the deep cleft made by the cuts than on a nearly flat terminal surface. This, however, is not due to any specific effect of the anterior tissue, but is merely a very general characteristic of wound-healing, not only in *Turbellaria*, but in many other forms, and is doubtless due to the fact that nutritive and other conditions for growth are better in such a cleft, where the growing parts are in contact on both sides with other tissue, than on surfaces where such contact exists only on one side.

I believe that the important point in connection with the problem of the influence of the central nervous system on regeneration in these forms lies in the question as to what constitutes the central nervous system. As Morgan states, the cephalic ganglia in the polyclads are enclosed in a definite sheath, but a further point of great importance which she does not consider at all is that the nerve roots contain numerous ganglion cells for a considerable distance from their point of origin in the ganglia. Reference to Lang's monograph of the polyclads[1] is sufficient to

[1] Lang, "Die Polycladen des Golfes von Neapel," *Fauna und Flora des Golfes von Neapel*, XI., Leipzig, 1884.

establish this point. There is then every reason to believe that the central nervous system comprises, not the ganglia alone, but the ganglia plus the nerve roots for a certain greater or less distance from their origin. Even when the ganglia are completely removed, the capacities of the central nervous system for regeneration and stimulation are not wholly lost, if sufficient portions of the nerve roots near the ganglia remain. In such cases the amount of regeneration is greater than when the nerve roots are also removed, and groups of eye spots may appear. In fact, in one case[1] I observed the regeneration of a small but distinct ganglionic mass after the apparently complete removal of the ganglia. It seems not improbable that if our technique were sufficiently exact to permit removal of the ganglia without injury to the nerve roots except at their origin, the regeneration even of the ganglia themselves, as well as of other parts, might be almost or quite as complete as when the ganglia remain.

L. V. Morgan's Fig. B2 of the regenerated anterior end of the nervous system after removal of the ganglia shows only fibrillar structure and she states that only fibrillæ are present in the mass. But when we recall the facts as to the histological structure of the nerve roots it seems extremely improbable that ganglion cells are totally absent from such regenerated masses. In all cases of the kind, which I have observed, some cells as well as the fibrillæ have always been present in the knot of tissue formed by the union of the nerve roots.

The development of eye spots in many of the cases without ganglia described by Morgan is undoubtedly due to regenerative processes in the remaining ganglionic nerve roots. In my own experiments I have found that in all cases, whatever the method of operation, where the ganglia plus a sufficient portion of the nerve roots are removed the regeneration is always slight, eye spots do not develop and the animal never shows any recovery from its sluggish unresponsive condition, *i. e.*, it behaves in all respects like a headless animal. On the other hand, where the roots are largely intact, regeneration is more rapid and proceeds farther, eye spots often appear and the recovery of motor activity and apparent spontaneity frequently occurs to a very marked extent.

[1]Child, "Studies, etc., VI.," Fig. 43, p. 526.

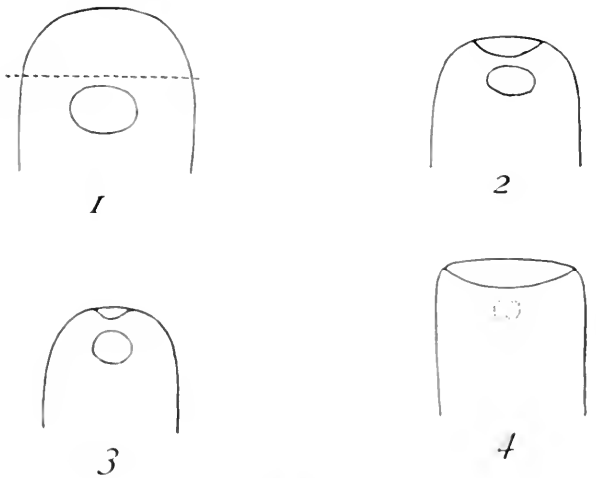
Since my later experiments confirm in all respects my earlier work, it seems unnecessary to describe them in detail and to figure all the various methods of operation and the results. I can only conclude that the apparent absence of effect of ganglionic removal upon regeneration in certain cases is due to one of two things, viz., failure to remove the ganglia completely, or the presence of the intact nerve roots. Morgan's experiments do not in any way prove that the central nervous system does not exert an influence upon the rapidity and amount, and so far as the sense organs are concerned, upon the character of regeneration in the polyclads.

As regards one point, however, Morgan's results as stated in her paper disagree so completely with my own that some further consideration is necessary. In the concluding paragraph of her paper the statement appears that "regeneration of the anterior tip of the worm, that is when the worm has been cut off anterior to the ganglia, occurs in the absence of the ganglia as well as when they are present." Individuals with the ganglia removed and the end cut off anterior to the ganglionic region regenerated as rapidly and completely as controls with uninjured ganglia and the anterior end cut off at the same level. In these experiments the ganglia were removed by using the cut end of a straw as a punch and after the wound thus made had healed the anterior region of the head was cut off.

I have performed this experiment a large number of times and on various species of Leptoplanidæ and with essentially uniform results, viz., that in all cases where the ganglia were actually completely removed, regeneration was less rapid and less complete than in control experiments with uninjured ganglia. Moreover the larger the portion of the nerve roots removed in addition to the ganglia themselves, the less rapid and less complete the regeneration. The operation is by no means easy to perform successfully and in many cases larger or smaller portions of the ganglia remain: such cases show all gradations from complete regeneration to a condition essentially like the pieces from which the ganglia are totally absent, but they must of course be regarded as unsuccessful experiments for our present purpose.

In my experiments the ganglia were removed with a straw in

the manner described above: two weeks later, after the wound had completely healed and the ganglionic region was filled in with new tissue, the anterior end of the head region was removed by a cut as indicated in Fig. 1. At the same time the anterior ends were removed at the same level from another series of individuals with uninjured ganglia.



FIGS. 1–4.

The condition of the animals without ganglia a week after the second operation is indicated in Figs. 2 and 3, while Fig. 4 shows the condition of the controls. The difference is marked and requires no comment. After two weeks more regeneration is almost or quite complete in the controls, while the animals without ganglia remain essentially as before and regeneration never proceeds further in them.

In these experiments great care was used to be certain that the ganglia were entirely removed. In various species the ganglia can be seen quite clearly from the ventral surface and examination from this side after the operation will usually show even rather small pieces of the ganglia if they remain. Morgan does not state how the total absence of the ganglia was determined in her experiments and it seems probable that in cases where the anterior end of the head regenerated as rapidly and as completely in animals supposedly without ganglia as in those with uninjured

ganglia some portions of the ganglia remained. It is possible that in some cases where the nerve roots were largely intact regeneration might be almost as rapid and complete as when the ganglia are present, but it is certainly impossible to make an extensive series of operations which are uniform in this respect. Morgan's experiments of this kind included however, only "several" worms.

The only conclusion possible seems to be then that the central nervous system, *i. e.*, the nerve roots near their origin from the ganglia, as well as the ganglia themselves, do affect in marked degree the rapidity and amount of regeneration of the anterior regions and, at least as regards the sense organs, its character as well. Moreover, where the ganglia, or the ganglia together with the nerve roots, are removed the method of operation makes no essential difference in the result. As most experiments, not only on the turbellaria but on other forms, indicate, it is probable that the early stages of the formation of new tissue are largely or wholly independent of the nervous system, but it is difficult to understand how the nervous system of an adult animal could fail to affect the amount and rapidity of growth in a regenerating part composed largely of muscles and sense organs. Absence of such an affect would be in direct opposition to the well established fact of the functional influence of the nervous system on various parts of the organism. The rate of metabolism and consequently the rate of growth—*i. e.*, provided nutritive material is present—in such parts must be in greater or less degree dependent upon nerve stimuli. Such an influence of the nervous system upon growth must, however, be sharply distinguished from the determination of differentiation of parts: the effect of the functional stimulus in the stricter sense is primarily quantitative rather than qualitative, so far as structure is concerned. These points were emphasized in my earlier papers.

Hull Zoölogical Laboratory,
University of Chicago,
October, 1910.

THE FORMATION OF GERM LAYERS IN ACTINIA BERMUDENSIS VERR.

LEWIS R. CARY,

PRINCETON UNIVERSITY.

While at the Bermuda Biological Station during the summer of 1909 I attempted to secure material for a study of the development of *Actinia bermudensis* Verrill, a viviparous actinian that is abundant between tide marks in the shaded limestone caverns along the shores of the smaller islands. Since this material proved to be lacking in the earliest stages in the development, so that the complete embryology could not be worked out, I have thought it advisable to publish the following account as it covers one of the most unsettled points in the development of anthozoans.

According to McMurrich (1891), the only well authenticated cases among the Cœlenterata in which the endoderm arises by invagination are those of *Nauthisoe* and *Pelagia*, both Scyphomedusæ. Jourdan's (1878) account of the formation of the endoderm in *Actinia aquina* he discredits on the basis of his own observations on *Metridium* (*marginatum*) *dianthus*. Kowalevskis's (1873) account of the process in *Adamsia rondeletti*, which in the original was inaccessible alike to Professor McMurrich and myself, he dismisses in the same manner saying that it was probably an error in interpretation.

Appellöf (1900) describes the endoderm formation in two species of actinians: *Urticina* (*Tealia*) *crassicornis* and *Actinia aquina*. His observations on the latter species confirm the opinion of McMurrich, namely, that there was no true invagination. In the case of *Urticina*, on the other hand, he describes and figures a true invagination, which from his figures could not be considered as an error of interpretation, since he worked with serial sections.

Faurot (1907) has described a true although rather unusual type of invagination in the development of *Sagartia parasitica* and *Adamsia palliata*.

In *Metridium*, according to McMurrich, the result of segmentation is the formation of a hollow blastula with a considerable cavity. Later the inner ends of the cells are constricted off—by the appearance of vacuoles in the line where the separation is to occur—to form the endoderm. At the time when this process is finished there appears at one pole of the blastula a slight de-

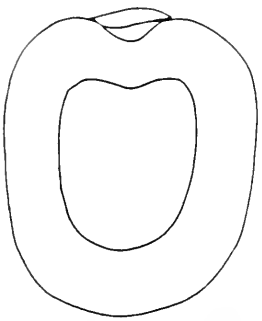


FIG. 1. Pseudogastrulation in *Metridium*. After McMurrich.

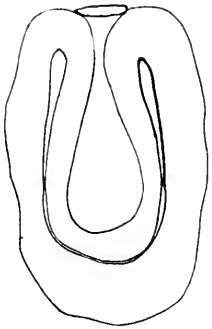


FIG. 2. Later stage in the pseudogastrulation of *Metridium*. After McMurrich.

pression which gives the embryo, when it is seen in optical section, an appearance similar to the early stages of a true invagination (Fig. 1).

When the mouth has broken through, the resemblance to an invaginate gastrula is even more complete, so that until such embryos had been seen in sections it would be almost certain to mislead any observer. In reality, however, the two layered condition had been reached before the mouth was formed. This so called "Pseudo-gastrula" McMurrich held to be the true condition in those forms in which invagination had been reported to occur.

All the material of *A. bermudensis* was obtained by slitting the adult individuals longitudinally, and then washing the embryos into a dish of sea water with the stream from a pipette. All stages including the young in which the second series of tentacles was complete, and which were ready to be liberated from the body of the parent were capable of swimming about actively by means of their cilia.

The earliest segmentation stages were never found among the

material obtained by washing out the adults, nor did sections of the mesenteries of the adult show any segmenting eggs, so the processes leading up to the formation of the blastula cannot be considered. In the section represented in Fig. 3, the blastula

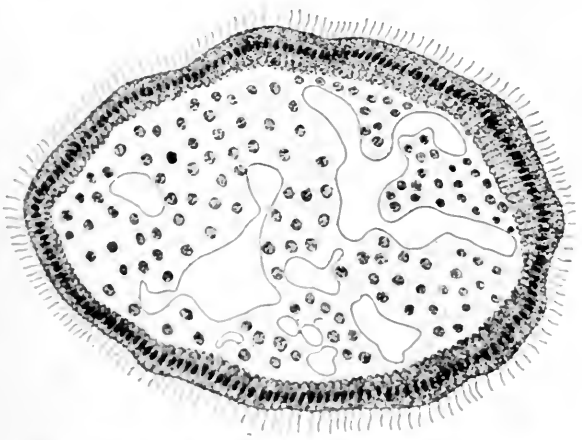


FIG. 3. Early blastula of *A. bermudensis*. Leitz 1/12 and no. 2 (reduced).

is completely formed. The cells are very numerous and of practically the same height over the entire circumference of the blastula. The interior of the blastula is to a great extent filled with a comparatively thin, lightly staining, plasma-like material, in which there are many yolk granules.

The manner in which the separation of the yolk spheres took place during the segmentation can be conjectured only, but, from the appearance of the stage just described it would seem probable that, just as in *Urticina*, there is never an extensive blastula cavity. Instead the yolk material is probably separated from the cytoplasmic portion of the cells in an early stage of segmentation.

In a later stage (Fig. 4) the blastula has become more elongated. The cells have become relatively thinner, and higher, while at one pole there is the first indication of the depression that marks the beginning of the infolding of one portion of the blastula wall to form the endoderm.

Within the interior of the blastula, there has been a marked increase in the relative amount of space unoccupied by nutritive

materials. The plasma-like substance has disappeared for a considerable proportion and the yolk spheres have undergone an apparent disintegration. Their outlines are no longer distinct, as in the earlier stages, and in many instances, they can be ob-

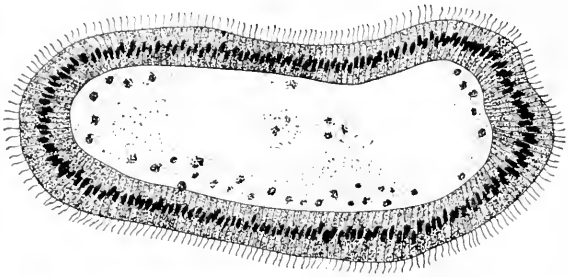


FIG. 4. Blastula of *A. bermudensis* just at the beginning of the invagination.

served breaking up into rather coarse granules which are being spread out through the surrounding plasma-like material.

In the stage shown in Fig. 5 the process of invagination has gone on until the section shows a well-marked early gastrula, and puts beyond any question the type of endoderm formation in this species. The character of the cells in the invaginating

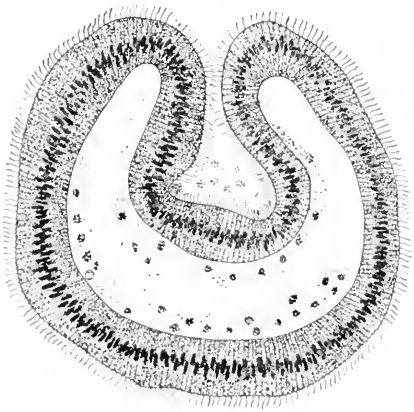


FIG. 5. Invagination of *A. bermudensis*.

area has not as yet undergone any change to distinguish them structurally from those making up the ectoderm.

The nutritive material within the original blastocœl shows practically the same conditions as noted for the stage last de-

scribed. Within the gastrula cavity there have appeared some few masses that are apparently composed of the rather coarse granules that come from the breaking up of the yolk spheres.

In the older gastrula, Fig. 6, almost all of the nutritive material has come to lie within the gastrocœl, only a comparatively few of the masses of granules, resulting from the disintegration of the yolk spheres, remaining in the original blastula cavity.

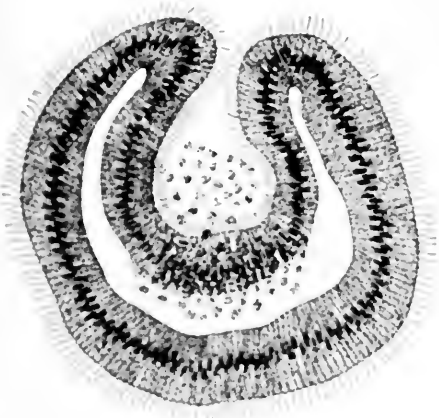


FIG. 6. Later invagination stage. *A. bermudensis.*

Appellöf (1900) describes the same sort of migration of the nutritive material during the invagination of *Urticina*. He raises the question as to whether the yolk material seen in the gastrocœl after the invagination is complete is the same as that seen in the blastocœl before invagination, and if so how the transfer could take place. Of the two possibilities in the way of a transfer he mentions: first that the yolk spheres might be absorbed by the cells of the invaginating layer and then be set free to collect in the gastrocœl; and secondly that the yolk elements pass ("sich drangen oder gedrängt werden") between the cells of the invaginating layer. According to his interpretation, his material supports the last mentioned view as many of the cells appear shrunken in diameter while yolk spheres may be seen between such cells separating their lateral walls one from another. He mentions besides that the walls at the inner ends of the cells are often very indistinct during the time when this process is

going on, although over the remainder of the gastrula wall there is no appreciable change in the characteristics of the cells.

Appellöf makes no direct mention, although his Fig. 13, Pl. 2, shows, that while the transfer of the nutritive material is taking place the yolk spheres are breaking up rapidly, so that to infer that the spheres pass through the invagination layer in their original condition is unnecessary. In the older gastrulæ of *A. bermudensis* a complete yolk sphere was scarcely ever found in the gastrocœl. While many of the masses of nutritive material still retained their identity and practically their original volume it could be observed in every instance, that the sharp outline was no longer apparent, and usually the granules were separated from one another. It is also noticeable in the section shown in Fig. 6, that nearly all of the yolk material present in the blastocœl is massed about the invaginating cell layer and that the inner ends of these cells are much less sharply defined than they were in the younger stages, Fig. 5, or than they are more laterally in the invaginating area. The central part of the invaginating area is more densely filled with granules than are those parts farther to the sides. The granules in this denser area are also markedly larger than the granules in the cytoplasm of the more laterally placed cells.

It seems, then, beyond question that in *A. bermudensis*, just as in *Urticina crassicornis*, there is an actual passing of the yolk material, in a practically unaltered state, through the layer of invaginating cells to the forming gastrocœl. As the cells of the invaginating layer approach those of the outer gastrula wall all of the yolk passes through so that the two layers come into contact and the supporting layer is secreted between them.

In an older embryo, Fig. 7, in which the formation of the stomodeum has begun, the gastrocœl—gastro-vascular cavity—still contains a considerable amount of the yolk material which now appears as distinct granules. In nearly all instances, however, the granules are arranged in groups which show clearly their origin from an originally more circumscribed mass.

In this last-mentioned stage the cells making up the endoderm have undergone a considerable change so that their histological characteristics are very different from those of the ectoderm cells

which have retained very nearly the appearance of the original blastula cells. The endoderm cells have now become much broader in proportion to their height, their cytoplasm is much less dense than formerly, and takes the stains less readily, while the nuclei have become markedly less conspicuous. In some regions, where the body of the embryo is most contracted, especially about the region of the forming stomodeum, the outlines of the endoderm cells are very indistinct, and indeed impossible to make out at the proximal ends of the cells.

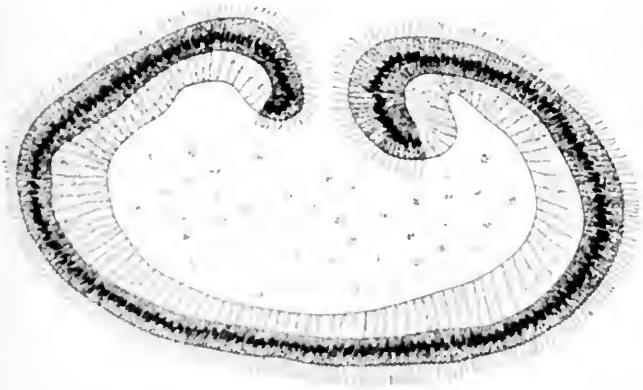


FIG. 7. Young embryo in which the stomodeum is forming. Leitz 7 and 2.

In regard to the interpretation of the optical sections shown in McMurrich's Fig. 10, Pl. XIII., it is interesting to compare my Fig. 6, with the figures of the embryos of *Actinia aquina* given by Appellöf. In the last mentioned form, where the endoderm arises by delamination, there occurs at the time of the breaking through of the mouth opening a decided thinning of both germ layers at the point where the mouth opening will appear. When the mouth has been formed the tissues are very thin all around it, and in a longitudinal section there appears about the mouth this thin area instead of the more thickened area which is found about the gastropore of an invaginate gastrula. So that in some instances at least, McMurrich's criticism of the interpretation of optical sections of whole mounts would not apply.

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