A STUDY OF THE SPERMATOGENESIS OF COPTOCYCLA AURICHALCEA AND COPTOCYCLA GUTTATA, WITH ESPECIAL REFERENCE TO THE PROBLEM OF SEX-DETERMINATION

BY

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WITH TWO PLATES

Very little work on the germ cells of the Coleoptera had been done until last year, when the form *Tenebrio molitor* was investigated by Dr. N. M. Stevens. The results obtained were so suggestive from the standpoint of sex-determination that she is at present engaged in examining a number of species of this order of insects.

The following paper is a contribution to this series of investigations, and I take pleasure in acknowledging my indebtedness to Dr. Stevens, both for material used and for direction in the work.

I. MATERIAL AND METHODS

The beetles under discussion are *Coptocycla aurichalcea* and *Coptocycla guttata*, both collected from *Convolvulus arvensis* at Woods Hole, Mass., September 5, 1905.

The testes were fixed in Flemming's strong solution, Hermann's platino-aceto-osmic, and Gilson's mercurio-nitric, each of which gave excellent results.

Heidenhain's iron-hæmatoxylin method was used for most of the preparations. Other stains, employed principally for chemical tests, were thionin, Delafield's hæmatoxylin and orange G, and with the material fixed in Hermann's and Flemming's fluids, the safranin-gentian stain of Hermann. The iron-hæmatoxylin slides were often counterstained with orange G.

II. OBSERVATIONS

The testes of these beetles consist of many follicles radiating from a central duct, the vas deferens, into which are poured the spermatozoa. We should expect then to find ripe spermatozoa at the inner end of the follicles and spermatogonia at the outer, with the intervening stages between. This is true in most cases but it is by no means an inviolable rule. Cysts containing ripe spermatozoa may be found mingled with those containing first or second spermatocytes, and second spermatocytes may occur in the same cyst with the first. The classification then cannot be made by the location but purely from the character of the cell itself.

1. *Coptocycla aurichalcea*

The equatorial plate of a spermatogonium of *Coptocycla aurichalcea* contains twenty-two chromosomes, most of which are V-shape, and radially arranged with the concave side outward (pl. I, Fig. 1). Near the center usually, though it may take any other position, is a very small chromosome, which is the small member of an "idiochromosome" group, and while we cannot here be sure of its mate we are safe in assuming it to be one of the five smaller chromosomes. An attempt was made to arrange the chromosomes in pairs according to form and size, but it soon became evident that they differed in different plates; the form, whether rod-shaped or V-shaped, depending largely on position with respect to the other chromosomes. As is unusual the behavior of the differential chromosomes in the spermatogonium is in every respect like that of the others: each divides longitudinally and passes to the poles, where condensed masses are formed.

The earliest spermatocyte shows a true condensation stage or bouquet stage [synizesis of McClung ('05)], in which the chromatin is in threads closely looped together at one side of the nuclear space (Fig. 2). The number of loops is here very evidently more than the reduced number and probably each loop corresponds to a spermatogonial chromosome. In a slightly later stage the loops straighten until many free ends are seen above the condensed mass (Figs. 3 and 5). Each of these strands appears to be a single

chromosome, and this idea is strengthened when in the next phase the distal ends are seen to bend toward one another in pairs and unite (Figs. 5 and 6). Fig. 4 is a cross-section of a nucleus at this stage. Thus synapsis takes place in the early part of the growth period soon after the last spermatogonial division. This agrees with Montgomery's ('03) description of the synapsis stage in *Amphibia* and furnishes more detailed evidence of the process.

Up to this period the idiochromosomes have been indistinguishable from the others, but a few very clear sections were obtained which showed at the base of the loops, close to the nuclear membrane, the larger member of the unequal pair (Fig. 5). This suggests the probability that the idiochromosomes retain their characteristic form during the greater part if not all of the synizesis and synapsis stages. All the chromatin has a strong affinity for stains during these periods, and the spireme is most distinct when first formed (Fig. 7), but almost immediately it becomes very faint (Fig. 8). The differential chromosomes still stain deeply, however. The spireme soon breaks up into separate rods, each showing a longitudinal split (Figs. 9, 10), even at this time there seems to be a characteristic form, one chromosome assumes a U-shape, one a triangular form, and one that of a nearly closed ring. These show still more clearly in the later prophase (Fig. 12).

The split disappears (Fig. 11), the chromosomes condense, growing slightly thicker and shorter (Fig. 12), until we get such forms as appear in Fig. 13. The centrosomes are large and distinct in this material, and at this stage two may be seen lying close against the nuclear membrane. In Fig. 13 we see them nearly 180° apart, and as yet no spindle fibers have appeared. Throughout the spireme stage the idiochromosomes are dense spherical bodies. In many cases only the larger member is visible (Figs. 7, 8, 9). In Fig. 11 both are plainly seen. In the later stages while the ordinary chromosomes are undergoing great changes the differential chromosomes remain the same (Figs. 12, 13).

An equatorial plate of the first spermatocyte (Fig. 15) shows eleven chromosomes, and since the spindle fibers are very large, these may often be seen in cross-section attached to the periphery of the chromosomes (Fig. 15). When the material is faintly

stained one chromosome stands out prominently, due to its retention of the stain (Fig. 14). This is the larger member of the differential group, as may be seen in a lateral view of the spindle (Fig. 21). The small idiochromosome fails to show here, due probably to the angle at which the pair is lying; in late prophase (Figs. 17, 22) the pair is easily distinguished. While in numerous metaphases the small chromosome is difficult to discern, there are a few cases in which it is clearly seen (Fig. 18). This unequal pair exhibits no difference in behavior during mitosis, but like the others, and at the same time, with the exception of one which always divides early (Figs. 18, 21), it divides transversely, the large member passing to one pole and the small member to the other (Fig. 25).

We have in this division one of the clearest cases of reduction division possible. In the spermatogonium the chromosomes are V-shaped. During the condensation stage they elongate into loops, at first closely massed together, but later these straighten and unite in synapsis to form longer loops. This is followed by the formation of a continuous spireme (Figs. 7 and 8) which soon breaks up into segments, each split longitudinally (Figs. 9, 10). In the prophase of the first spermatocyte many of the chromosomes are thick rods, which upon examination are seen to be two V's joined end to end, giving in profile a typical E-shape (Fig. 16, *e*). The spindle fiber is attached at the vertex of each V of the bivalent chromosome and in anaphase this element separates at the original place of union, at right angles to the initial longitudinal split (Figs. 18, 20). The V-shaped chromosomes observed in the spermatogonium are thus restored and carried into the second spermatocyte. Exactly the same thing is true in the separation of the components of the cross-shaped chromosome (Figs. 16, *a—d*), and of the ring-shaped one (Fig. 16, *l—o*, and Figs. 22, 23, 24).

There is no rest stage between the two generations of spermatocytes, but almost immediately the massed chromatin in the late anaphase (Fig. 26) separates into chromosomes which begin to arrange themselves in the equatorial plate (Figs. 27, 28, 29). In Fig. 30 we see this in progress, the small chromosome being very distinct. In Figs. 27 and 28, we again see eleven chromosomes

of which one is the small idiochromosome. Having seen that in the first division the large idiochromosome goes to one pole and the small to the other, we should expect to find half the daughter cells containing the large and half the small chromosome of this pair. This is exactly the case. Fig. 29 shows a representative of the cells possessing the large member. This is especially clear in material very much destained, for here as in earlier stages this peculiar chromosome holds the color much longer than the others. In other respects the differential chromosomes are not different, but divide longitudinally with the ordinary chromosomes.

In early anaphase each V shows a distinct split which lies in the plane of the equatorial plate (Fig. 31). That this is the original longitudinal split seen in the early prophase (Figs. 9, 10) there can be no doubt. One chromosome which maintains its identity from the early prophase through the first spermatocyte as a cross is sufficient evidence. In the late prophase and even in metaphase this chromosome (Fig. 16, *a—e*) may be seen to be longitudinally split (Fig. 16, *d*). The line of separation comes at right angles to this split and the univalent chromosomes pass to the poles as V's, each possessing a longitudinal split, though it closes and cannot be seen again until the beginning of the following anaphase. The chromosomes are typically arranged with the vertices attached to the fibers and pointing inward. In division they are drawn apart, at first with the ends of the V's pointing poleward (Figs. 31, 32) but later the vertices turn toward the poles, though disorder often reigns until a comparatively late anaphase (Fig. 33).

In the spermatids there is a great amount of archoplasm, and in the late telophase of the second division it occupies the greater part of the cytoplasmic area (Fig. 36). At a slightly later stage the mass loses entirely its fibrillar structure and, condensed somewhat, lies as a gray sphere (iron hæmatoxylin) against the nucleus (Fig. 38). This is the so-called *nebenkern* of the spermatid. The entire cell now elongates, and with it the axial filament. The archoplasm has assumed the form of a large pennant attached to the nuclear membrane, and the axial fiber runs throughout its length and often beyond (Fig. 39).

A cross-section of the tail at this time shows that the archoplasmic sheath is folding about the axial fiber (Fig. 50, *a*). When the transformation is nearly complete the two cannot be distinguished from each other, the sheath lies close against the filament with a thin layer of cytoplasm outside (Fig. 50, *c*). Often in cross-section the sheath appears to be split (Fig. 50, *b*). Since longitudinal views of the tails reveal nothing of the kind we are left to conjecture that this appearance is due to oblique sections of the trough-shape sheath. The fact that many of these appear together does not alter the explanation, for the spermatozoa and late spermatids lie parallel in large numbers.

There is one peculiar but apparently typical stage in the development of the tail: while the head is yet round, though the chromatin is much condensed, the axial filament has assumed a vacuolated appearance (Fig. 40). This lasts until the head has begun to elongate, and is just over in Fig. 41. This is not to be confused with the rare occurrences of double filaments seen in Fig. 48. These are the products of giant cells, due to a failure of one of the divisions of the spermatocytes or the spermatogonia to complete itself. They consequently have two centrosomes and two axial filaments. Paulmier ('99) found similar conditions in *Anasa*. As in other insects the axial filament in the Coleoptera seems to arise in close relation with the centrosome.

After the vacuolated stage the tail narrows and lengthens much more and we see it in its final form in Fig. 44. All this time the head of the spermatid has been changing. The dense chromatin mass begins to lose its affinity for hæmatoxylin until a deep gray results. A structure is now revealed in the nucleus, not heretofore seen, a small densely staining nucleolus-like body (Fig. 36). The nucleus now widens its circumference and the chromatin condenses around the membrane in the form of a ragged border (Fig. 38). The nucleolus-like formation is not seen at this stage, but the supposition is that it is merely obscured by the dense patches of chromatin, for in the next stage it again appears and occupies a most characteristic position: the chromatin is arranged in a crescent shape and the nucleolus lies in a clear area between the arms of the crescent (Fig. 37). The chromatin becomes diffuse and min-

gles with the karyolymph until a uniform light gray results (iron hæmatoxylin). The nucleolus is unaffected, changing neither in size nor in staining reaction. It makes a change, however, in position about this time, moving from a point opposite the centrosome around the periphery of the nucleus until it often lies very near the centrosome (Fig. 49).

The head of the spermatid lengthens and becomes vacuolated (Fig. 41), but as the elongation increases the vacuoles disappear and the deeply staining nucleolus-like body is apparent, lying closely against the nuclear membrane (Fig. 45, *y*). Very soon this single body breaks up into two, three or four parts and later, it disappears (Figs. 46 and 47). The head condenses, lengthens, stains intensely (Fig. 42), and in its final form is spirally twisted, resembling the *Bacterium spirillum* (Figs. 43, 44).

It is impossible at the present time to say just what may be the function of this peculiar, deeply-staining spermatid element. It suggests the accessory of Orthoptera which can be seen in one-half the spermatids (McClung, '99, '00, '02a), and at first it seemed probable that it might be one of the idiochromosomes in the beetle as it, in one stage, is about the size of the larger member of the pair. Instead of being found in half the cells, however, it is without doubt in all, so that this explanation was relinquished. It is characteristic of the spermatids of all the beetles thus far studied and of some of the Orthoptera.

2. *Coptocyclus guttata*

In external appearance *Coptocyclus guttata* is very different from *C. aurichalcea*, but in their germ cells there are many points of resemblance. *Guttata* has the smaller number of chromosomes, eighteen in the spermatogonia instead of twenty-two, but the size and form are much the same in the two species (Figs. 1 and 51). The unequal pair is also present here.

The suggestion that synapsis takes place immediately after the condensation stage in *C. aurichalcea* is strongly confirmed by *guttata*. In the condensation or synizesis stage, the chromatin is in

the form of loops with the ends against the side of the nuclear membrane (Fig. 52). This point Montgomery calls the distal pole of the nucleus and the opposite point on the nuclear membrane, the central pole. The process is the same as in *aurichalcea*; the loops straighten, thrust the free ends toward the central pole (Fig. 53), bend toward each other in pairs and unite end to end (Fig. 54). The end of the chromatin thread either bears an enlargement or stains more deeply in cross-section for there is at this point the appearance of a deeply staining bead. This dark spot thus conveniently marks the place of union in the bivalent chromosomes, and we are led to the conclusion that the short loops first observed in the synizesis stage are univalent chromosomes. The idiochromosome pair is very distinct at the base of the loops as shown in Fig. 52.

The growth stages are not unlike those of *C. aurichalcea*. A spireme (Fig. 55) is formed which varies in its staining reactions exactly as in *C. aurichalcea*. The thread finally breaks up into bivalent elements which stain deeply and exhibit a longitudinal split, and at last condense into forms which they retain during the first division. In Fig. 57 are seen two bivalent chromosomes assuming the form of rings, and in Fig. 58 these have closed together. This figure also exhibits crosses which later change to the form found in *aurichalcea*. Most of the chromosomes of this species are dumb-bell-shaped in the first spermatocyte mitosis and of the usual V-shape in the second. The idiochromosomes maintain their spherical form throughout all the stages.

An equatorial plate of the first maturation division shows nine chromosomes arranged usually in the order seen in Fig. 60, with seven in a circle about the other two. All nine chromosomes may be seen in lateral view in Fig. 59, where they are just coming into the spindle. The dumb-bell shape of four is here quite evident and the unequal pair is conspicuous. Three of the others appear as straight rods and the other one bent in V-form.

During metaphase the chromosomes arrange themselves with their long axes parallel with the axis of the spindle, and later they divide at right angles to their length. In other words, they exhibit qualitative division, bivalent chromosomes separating at the point

of union made during synapsis. As a result the small chromosome of the idiochromosome pair goes to one pole while the large member goes to the other (Fig. 63). The equatorial plates of the second division confirm this, half possessing nine V-shaped chromosomes of approximately equal size (Fig. 71), the other half having eight large and one small chromosome (Fig. 65). Fig. 66 shows the second maturation spindle with this small chromosome in metaphase, and in Fig. 64 it is seen dividing.

Here, then, as in *C. aurichalcea* and *Tenebrio molitor* (Stevens, '05) half the spermatids will possess the small idiochromosome, and half the large.

The transformation of the spermatid is essentially the same as in *aurichalcea*. The deeply staining nucleolus-like body is present but no clue to its function or nature is given. The ripe spermatozoa are not spirally twisted but resemble that stage of *aurichalcea* seen in Fig. 42.

Owing to lack of material it was impossible to study the dividing somatic cells of the male and female forms. That they would exhibit the same conditions found repeatedly in other beetles there is no doubt. By permission I have reproduced four drawings from Dr. Stevens' originals, which show the chromosome of (Fig. 67) a somatic cell from the digestive tract of a male pupa of *Tenebrio molitor*, and (Fig. 68), a female somatic cell of the same form found in the egg follicle; (Fig. 69) a female somatic cell of *Trirhabda virgata* from the egg follicle, and (Fig. 70) a male somatic cell taken from the larval body of the same species.

Here, as in other forms that Dr. Stevens has investigated, the small idiochromosome goes to the male and the large one to the female.

SUMMARY OF OBSERVATIONS

Coptocyclus aurichalcea

1 The spermatogonial number of chromosomes in *Coptocyclus aurichalcea* is twenty-two, twenty-one of which are V-shaped and one very small one, spherical in form.

2 Synapsis takes place in a manner, so far not described but also observed in certain beetles now being investigated by Dr. N. M. Stevens. The loops seen in the synizesis stage, which represent individual chromosomes, straighten and unite in pairs by the free ends which are pushed up into the nuclear space. Pseudo-reduction, therefore, occurs just after synizesis and just before the formation of the spireme.

3 The first maturation mitosis is a transverse, reducing division, the second longitudinal, occurring along a lengthwise split formed early in the prophase.

4 There is present a typical pair of idiochromosomes [according to Wilson's definition ('05 and '06)] which, with the others, divides qualitatively in the first division and quantitatively in the second, the small member, therefore, going to one-half the spermatozoa and the large member to the other half.

5 The chromosomes show marked individuality from the beginning of the prophase, one having the form of a ring, two of a cross, several the shape of an E, and finally the unequal pair.

Coptocycla guttata

1 The spermatogonial number of chromosomes is eighteen, seventeen large V-shaped chromosomes and one which is small and spherical.

2 All other observations on this species confirm those on *aurichalcea*.

GENERAL DISCUSSION

Individuality of the Chromosomes

Convincing results have been published in regard to the individuality of the chromosomes by Boveri ('02), who found a difference in their function; by Sutton ('02), who found a difference in size; and by Baumgartner ('04), and others, who have discovered a difference of form.

The idiochromosomes indicate so clearly a difference in function as well as in size that it is unnecessary to go into detail on this

point. The ordinary chromosomes also confirm the size difference; though no careful measurements were made, it is obvious that such differences exist at least in most cases (Fig. 19). However, the difference in form is most evident in *Coptocycla aurichalcea*, and furnishes another strong support for the doctrine of the individuality of the chromosomes.

Of the eleven bivalent chromosomes several possess forms characteristic enough to mark them as the same in different generations; there are two or three crosses, several E's, a ring and an unequal pair (Fig. 16). There is, doubtless, a size difference that separates those of a group though this is not as obvious in some cases as in others. The ring form occurs only once in this species, but twice in *guttata* and maintains its identity of shape from an early prophase, being much more expanded then, however, than later. It is formed by the V-chromosomes of the spermatogonium uniting by both ends, as is plainly shown in Fig. 16, *l*, where two ends are not firmly joined. In a late metaphase the bivalent pair seems to elongate slightly; this closes the opening and gives the appearance seen in Fig. 23. In profile the ring assumes an oval form, with the central opening much smaller (Fig. 16, *n*), but this is distinguished from the actually elongated phase by the length (Fig. 23).

Mendel's Law

Assuming that the idiochromosomes are sex-chromosomes, or represent the sex-characters, they furnish excellent opportunities for speculation on the application of Mendel's law to chromosomes.

If the Mendelian principles of segregation apply to sex, there should be in the second generation 25 per cent males, 50 per cent hybrids, and 25 per cent females. Castle ('03) concludes that there are no individuals pure in regard to the sex-character, but only hybrids are produced. Now to apply this theory to the beetles. We know by actual observation that the male somatic cells possess the small idiochromosomes, and the female somatic cells the large one. Below are given in a schematic representation

two kinds of eggs and two kinds of spermatozoa with the dot above to indicate the idiochromosome and the letter to show the sex character.

There are four possible combinations here:

Now by actual examination of the male and female somatic cells we know that no such combinations as (2) and (3) exist. Our observations, therefore, strongly support the theory of selective fertilization, only gametes bearing opposite sex-characters fusing.

While the beetle furnishes no explanation why femaleness as a character often dominates (Castle '03) yet its chromosomes indicate why it is recessive a part of the time in these insects. In the 50 per cent of spermatozoa that carry the female character in the form of the small idiochromosome, this is overpowered by the much larger male idiochromosome in the eggs with which they unite. When the chance is even, *i. e.*, when the idiochromosomes are equal in size, then femaleness invariably dominates.

The Idiochromosomes

But two groups of animals thus far investigated are known to possess the idiochromosomes; these are the Hemiptera and the Coleoptera. It is probable that they exist in other forms and have been wrongly classified as was the case with Paulmier's ('99) work on *Anasa*, and Montgomery's ('01) on *Cœnus delius* and *Euschistus tristigmus*. Paulmier failed to distinguish between the accessory and the microchromosomes, while Montgomery saw the small idiochromosomes in the resting stages and the different number of chromosomes in different cysts, but misinterpreted them.

To Wilson is due the correct interpretation of these idiochromosomes in the Hemiptera heteroptera, as a distinct pair of chromosomes with a definite and characteristic behavior in the different generations.

Slightly earlier than this and independently Dr. Stevens working on one of the Coleoptera, *Tenebrio molitor*, found an unequal pair of chromosomes, which proves upon comparison to be essentially the same as those of the Hemiptera.

In details of behavior, however, these chromosomes differ markedly in the two groups. As in *Lygæus* and *Cœnus* (Wilson, '05) the idiochromosomes of *Coptocyclus* maintain their identity throughout the growth period. They are first distinguished in the synizesis stage as a compact, spheroidal body at the base of the chromatin loops (Fig. 52). The small member has not been observed at this stage, but it is doubtless present and is obscured by the larger of the idiochromosome pair. As this pair appears in the spheroidal form, so early in the growth stage, it is a question whether it ever assumes the form of loops with the other chromosomes.

In *Lygæus* in the synizesis stage the larger differential chromosome is elongated and bent U-shape. In the post-synaptic phase instead of lengthening and splitting longitudinally as in *Lygæus*, both of the idiochromosomes of *Coptocyclus* maintain their compact, rounded form. In early prophase these chromosomes in Hemiptera exhibit a bipartite structure and sometimes remain separated while the ordinary chromosomes have fused in synapsis.

In *Coptocycla* the unequal pair has fused, but neither member shows any tendency toward being bipartite. In fact, at no time is there a hint of a longitudinal split, unless the vacuolated appearance of the large idiochromosome in the early growth stage may be considered as such.

The later behavior of the idiochromosomes is markedly different in the two groups. In the Hemiptera they remain separated and univalent in the first maturation mitosis, but at the close of this division their products conjugate to form a dyad, which in all but one form, *Nezara*, is asymmetrical. In the Coleoptera the behavior during mitosis is exactly like that of the ordinary chromosomes; having united in the synapsis stage to form a bivalent, they divide transversely in the first mitosis and longitudinally in the second. The result is, of course, the same in both cases; the spermatids are of two kinds as regards the idiochromosomes, half possessing the small and half the large one. The distribution of these chromosomes to the somatic cells of the two sexes is also the same in the two groups; the male cells contain the smaller, the females cells the larger idiochromosome.

Sex-determination

The observations recorded in the present paper add nothing new to the subject of sex-determination, and their chief value consists in their confirmation of the very suggestive work on *Tenebrio molitor* (Stevens, '05).

Since McClung ('00) advanced his theory that the accessory chromosome of the Orthoptera is a sex-determinant, numerous investigators have sought evidence for or against it. The first in its favor was that of Sutton ('02), who found the odd chromosome present in the male somatic cells and absent in the female cells of *Brachystola magna* (23 in ♂ cells, 22 in ♀ cells). Since that time, however, Wilson has found the reverse true for Hemiptera, *i. e.*, the additional chromosome in the female somatic cells (*Anasa* 21 ♂, 22 ♀), and this leads him to question Sutton's count for the Orthoptera. Wilson suggests that the accessory chromosome of *Anasa*, *Protenor* and the Orthoptera is the homologue of the larger

member of the idiochromosome group found in *Cœnus*, *Lygæus* and certain other Hemiptera, and its missing mate is the homologue of the small idiochromosome. He thus supports the view of Paulmier and Montgomery in regard to degenerating chromosomes.

Knowing from his observations on *Anasa* that the accessory goes to the female in the Hemiptera, he, therefore, conjectured that the larger of the idiochromosomes would be found in the female somatic cells, and the small one in the male somatic cells, and he later found positive proof of this.

As stated before Dr. Stevens while investigating a form of beetle, *Tenebrio molitor*, found that an unequal pair of chromosomes is present, the large one of which goes to the female somatic cells, and the small one to the male somatic cells. Since then she has confirmed this in other species of Coleoptera, so that at last there seems to be much indisputable evidence for the chromosome-sex-determinant theory.

The question arises, what is to be done with such forms as *Thermopsis* (Stevens, '05) and *Banasa* (Wilson, '05). In the former there are no chromosomes which have any external peculiarity that would mark them as sex-determinants; in the latter there are two sets of chromosomes that we interpret as sex-determinants when they appear separately in other forms.

The solution for *Thermopsis* is perhaps less difficult than for *Banasa*; one pair of the chromosomes may bear the sex-character although there are no external differences. This does not conflict with present views. In *Banasa*, the situation is different. Here we have a pair of idiochromosomes as well as a typical accessory. In other cases Wilson has suggested that the large idiochromosome is the homologue of the accessory inasmuch as both are members of pairs, one member of which is degenerating or has already disappeared. This seems plausible and if taken from the standpoint of degeneration only, there is nothing conflicting in the fact that two pairs of chromosomes even in the same cell are undergoing the change. In fact, as Wilson suggests, it adds weight to the idea of degenerating chromatin, for we have no reason to suppose that degeneration is necessarily limited to one pair.

However, taken from the standpoint of sex-determination it seems to offer serious difficulties. While we are perhaps not justified in assuming that the sex character is confined to one chromosome, or to one pair of chromosomes, all recent observations point that way; then on *a priori* grounds it seems improbable that the same function should be assigned to two pairs of chromosomes in the same cell. With the numerous characters to be transmitted it would seem more likely that one pair of chromosomes must be the bearer of many qualities.

In *Banasa* there are four classes of spermatozoa which contain:

- (1) the small idiochromosome + the accessory;
- (2) the large idiochromosome + the accessory;
- (3) the small idiochromosome — the accessory;
- (4) the large idiochromosome — the accessory.

If the chromosome relations in the male somatic cells of *Banasa* correspond to those in *Lygæus* and *Anasa*, only one of these classes of spermatozoa (3) can be used. Any combination of (1), (2), (4) would refute the idea of homology of these two types of chromosomes. The same thing is true as to the production of females; but one kind of spermatozoon (2) can be functional.

This means that three-fourths of the spermatozoa are functionless as regards production of males, three-fourths as regards production of females and one-half absolutely functionless. Such a condition seems most improbable, but, of course, the only way of determining it is to study the male and female somatic cells of *Banasa*. This promises interesting results as it will either refute the homology theory (for this form, at least) or reveal new facts in regard to selective fertilization and a functional and non-functional condition of spermatozoa.

While Castle believes there is no hard and fast rule for dominance in the sex character in dioecious individuals, it seems that in the beetles we have a clear case of female dominance. In cells where the male and female sex chromosomes are equal in size, femaleness invariably dominates. Where they are unequal (the female small, as in Coleoptera and some Hemiptera, or entirely missing as in Orthoptera) then the female character is visibly

recessive, due merely to its reduced strength or entire disappearance in the male.

The results pointed out for *Tenebrio* and confirmed in this paper are briefly as follows: an unequal pair of chromosomes is present which, we have strong evidence for believing, transmit or determine the character of sex. The fact that the small one invariably occurs in the male somatic cells and the large one in the female somatic cells seems on first thought to mean that the small one carries maleness and the large one femaleness. But Wilson's thorough analysis of the conditions in Hemiptera ('06) has shown that the larger idiochromosome which alternates between the sexes must bear the male character, while the small idiochromosome, which is confined to the male sex, must represent the recessive form of the female character.

While it has been proved beyond doubt that certain chromosomes are concerned in the determination of sex among insects, a general application of the theory cannot yet be made, but it must for the present be limited to those forms possessing the accessory or the idiochromosomes. Without doubt further investigation will reveal either similar differential chromosomes in all forms, or show something homologous to them. It may not be a difference of size or shape that will distinguish them from the ordinary chromosomes, but probably one of behavior. Even in case the last difference is not found it need not disprove the chromosome-sex-determinant theory, for the sex character doubtless can be carried like other pairs of antagonistic characters without affecting the chromosome visibly. The eggs of comparatively few forms have been investigated, and it is possible that a dimorphism of the chromosomes in the matured oocytes may be found in certain groups.

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DESCRIPTION OF PLATES

All drawings were made with the aid of a camera lucida. A Zeiss oil-immersion 2 mm. objective and oc. 12 were used throughout, and the drawings have been reduced one-third.

PLATE I

Coptocyclus aurichalcea

- Fig. 1. Equatorial plate of spermatogonial mitosis, 22 chromosomes.
- Fig. 2. Bouquet-stage or synizesis, showing univalent chromosomes in loop form.
- Fig. 3. Later stage in which loops are straightening.
- Fig. 4. Cross-section of the stage in Fig. 2.
- Fig. 5. Synapsis. Fusion of univalent chromosomes end to end at y . Large idiochromosome α , at base of loops.
- Fig. 6. Late synaptic stage, showing the relative lengths of the bivalent elements.
- Fig. 7. Formation of spireme; α , the large idiochromosome.
- Fig. 8. A slightly later stage; spireme very pale, α deeply stained.
- Figs. 9, 10. Very early prophase, showing the longitudinal split in the chromosomes, idiochromosome excepted.
- Fig. 11. Still later prophase. Both members of the unequal pair visible (α).
- Figs. 12, 13. Prophase in which the chromosomes are contracting into their characteristic forms.
- Fig. 14. An equatorial plate of the first division showing nine of the eleven chromosomes. The idiochromosomes retain the stain much longer than the others.
- Fig. 15. An equatorial plate of the first mitosis with the full number, eleven chromosomes, present. The large linen fibers are seen attached to the chromosomes.
- Fig. 16. Three types of chromosomes, found in *C. aurichalcea*, from various points of view, $a-e$, the cross-shaped chromosome; a , between a front and side view; b , front view when the longitudinal split is closed; c and e , profile; d , front view showing split, $f-h$, the E-shaped chromosome f , front view; g , profile, bivalent chromosome dividing; h , profile; $i-o$, ring-shaped chromosomes: i , the two univalent chromosomes not well fused; m , probably later stage in which union is more complete; n , ring closed and seen in profile; o , central opening growing smaller.
- Fig. 17. Late prophase of first spermatocyte; chromosomes coming into equatorial plate; α , the idiochromosome pair.
- Fig. 18. Equatorial plate formed and one chromosome divided ahead of the others.
- Fig. 19. Shows the size difference in some of the chromosomes.
- Fig. 20. Anaphase of the first division, showing distinctly the form of the univalent chromosomes, and the manner of separation.
- Fig. 21. Side view, first spindle, showing the densely staining idiochromosome when the others are very pale.
- Figs. 22-24. Different views of the ring chromosome.
- Fig. 25. Idiochromosomes dividing in first mitosis; l , large chromosome; s , small chromosome.
- Fig. 26. Late anaphase of first spermatocyte.
- Figs. 27, 28. Equatorial plates of second mitosis, showing 10 large and one small (s) chromosome.
- Fig. 29. Equatorial plate of same mitosis, showing 11 large chromosomes, l , the large idiochromosome.
- Fig. 30. Prophase of second mitosis, showing 10 large chromosomes and the small idiochromosome.
- Fig. 31. Anaphase in which the large idiochromosome is dividing.

PLATE II

Coptocycla aurichalcea

Fig. 32. Anaphase of second maturation division, showing the position assumed by the chromosomes when first separated.

Fig. 33. Late anaphase; small idiochromosomes dividing.

Fig. 34. Late anaphase; unusual case in which the form of the chromosomes is not obscured by massing.

Fig. 35. The usual late anaphase.

Fig. 36. Very early spermatid showing a great amount of archoplasm.

Fig. 37. Nucleus at a slightly later stage, showing the peculiar nucleolus-like body.

Fig. 38. Archoplasmic substance in the form of a sphere applied to nuclear membrane.

Fig. 39. Archoplasm elongated into a pennant form with axial filament running throughout.

Fig. 40. Vacuolated stage in formation of the tail.

Fig. 41. Vacuolated appearance of head *c*, centrosome.

Figs. 42-44. Later stage in the transformation of the head. Ripe spermatozoa (44) possess a spirally twisted head.

Figs. 45-47. Later behavior of the nucleolus-like body.

Fig. 48. Giant spermatozoa, with two centrosomes and two axial filaments.

Fig. 49. Shows the migration of the nucleolus-like body.

Fig. 50. Cross-sections of the tail of the spermatid, *a-b*, at about the stages shown in Fig. 41; *c*, in Fig. 44.

Coptocycla guttata

Fig. 51. Equatorial plate of spermatogonial mitosis; 18 chromosomes.

Fig. 52. Synzesis stage showing univalent chromosomes in loop form. Large idiochromosome at base of loops.

Fig. 53. Later stage in which loops are straightening and bending toward each other.

Fig. 54. Synapsis. The dark bead at the highest part of loop marks point of union.

Fig. 55. Spireme stage; chromatin very pale with exception of unequal pair (*x*).

Fig. 56. Same stage, showing small idiochromosome somewhat removed from the large member and connected with it by a chromatin strand.

Figs. 57, 58. Formation of ring and cross-shaped chromosomes.

Fig. 59. Late prophase in which all nine chromosomes are seen from a side view. *x*, the idiochromosome pair.

Fig. 60. Equatorial plate of the first maturation mitosis; 9 chromosomes.

Figs. 61, 62. Prophase and metaphase, respectively, showing the idiochromosome pair (*x*).

Fig. 63. Anaphase of first mitosis; the idiochromosome pair, dividing qualitatively. *s*, small idiochromosome; *l*, large.

Fig. 64. Very early anaphase of second mitosis, showing division of the small (*s*) idiochromosome.

Fig. 65. An equatorial plate of the second spermatocyte in which the small idiochromosome (*s*) is present.

Fig. 66. Metaphase of second spermatocyte. The small idiochromosome (*s*) undivided.

Fig. 67. Equatorial plate from dividing somatic cell of male pupa (*Tenebrio molitor*), showing nineteen large and one small chromosome.

Fig. 68. Equatorial plate of a dividing cell of follicle of a young egg (*Tenebrio molitor*), showing twenty large chromosomes.

Fig. 69. Equatorial plate of a dividing follicle cell of a young egg (*Trirhabda virgata*), showing twenty-eight large chromosomes.

Fig. 70. Equatorial plate from the somatic cells of a male larva (*Trirhabda virgata*), showing twenty-seven large and one small chromosome.

Fig. 71. An equatorial plate of same spermatocyte in which all nine chromosomes are of approximately equal size.