# AUTOSOMAL ELIMINATION AND PREFERENTIAL SEGREGATION IN THE HARLEQUIN LOBE OF CERTAIN DISCOCEPHALINI (HEMIPTERA)

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## Introduction

It has been known for some time that the various lobes in the testis of many species of pentatomid Hemiptera show constant differences in the size of their cells. Bowen (1922a and b) who investigated this condition most recently, concluded that it is attributable mainly to differences in the volume of cytoplasmic elements. That is a finding which I can only confirm; if differences in the volume of chromatin exist they must be very small. However in some species there is a testicular lobe whose cells differ from those of other lobes not only in size but which has also evolved an entirely novel process of maturation. The main features of this maturation are in several instances almost fantastic in character, and the evolution and constant occurrence of such "harlequin" lobes is a matter of some interest.

It should be emphasized that we are dealing here not with accidental or sporadic occurrences. In the species concerned the harlequin lobe is found in each testis of every male. Moreover for any given species it is always a certain and very definite lobe that is thus characterized (in the Discocephalini here treated it is the fifth) and hence it is clear that its development involves conditions that are fundamentally and firmly established in the species as it is now constituted.

Harlequin lobes have so far been encountered in three species of Loxa, a species of Mayrinia (Schrader, 1945a and b), and in a species of Brachystethus (Schrader, 1946). In the last named the departure from a normal meiosis lies primarly in the autosomes which are shunted out of the spindle in both divisions; in Loxa and Mayrinia the aberrancy takes the form of amitosis and fusion in the spermatocytes, resulting in a highly variable heteroploidy. The meiotic anomalies of Brachystethus and Loxa thus appear to be in no way related and yet it seems only natural to assume as a working hypothesis that the evolution of harlequin lobes involves similar basic conditions in all the species involved.

It is likely that further investigations will discover that harlequin lobes are present in a great many species. To the five species mentioned above and the three taken up in the present paper may be added at least three further species which I have not as yet fully analyzed—a total of eleven. Taxonomically speaking, these species cover a wide range. Loxa and Mayrinia represent typical genera of the tribe of Pentatomini; Brachystethus is so closely related to the Edessini as to furnish almost a "bridging" genus between that tribe and the Pentatomini; and the Discocephalini constitute a tribe so distinct from the other pentatomid tribes that it has sometimes been elevated to the rank of a subfamily (Lethierry and Severin, 1896).

Conditions in the females of all these species are still unknown, except as they were used in all instances to check the identification of the sex chromosomes in the males. Cytologists need hardly be told that this gap in our knowledge is due mainly to the technical difficulties that render a study of meiosis in the egg so onerous a task.

I should like to point out again as I have done in my study of Brachystethus, that the investigation of the harlequin lobe is made under the almost ideal conditions of a natural experiment. The adjoining lobes of the same testis are perfectly normal and serve continually as a control; frequently the normal and aberrant cells can be studied in one and the same field of the microscope.

## Material and Methods

The Discocephalini investigated are: *Mecistorhinus melanoleucus* Westwood (one male from Panama); *Mecistorhinus tripterus* Fabricius (four males and two females from Costa Rica); *Mecistorhinus sepulcralis* Fabricius (one testis each from eight different males and the ovaries from one female, all from Piracicaba, Brazil); *Neodine macraspis* Perty (five males from Costa Rica); and *Platycarenus notulatus* Stål (three males and one female from Costa Rica). The last named species has no harlequin lobe and is only briefly mentioned in the following pages.

My thanks are due to the eminent hemipterist, Mr. H. G. Barber, who identified all the species of Mecistorhinus. To Professor S. de Toledo Piza of the University of São Paulo, Brazil, I am deeply indebted for the material of *Mecistorhinus sepulcralis*.

Fixation was made in either Bauer's convenient modification of Allen's Bouin or in Sanfelice. As in all my recent studies of mitosis, I have employed three staining methods. The Feulgen technique is indispensable as a test for chromatin; gentian violet (in Smith's modification of Newton's method) is often very useful for a study of the detailed structure of the chromosomes but even when combined with erythrocin is not an efficient stain for the spindle apparatus in pentatomids; whereas Heidenhain's hematoxylin remains beyond all comparison the best means for bringing out asters, centrioles and spindle fibers. As noted above, female material was studied only to check identification of the sex chromosomes in the male.

In all the Discocephalini where a harlequin lobe occurs, it is the fifth of seven lobes in each testis. In every case it is two or three times as voluminous as any other lobe although its spermatocyte cells after the leptotene stage are smaller than those of the rest of the testis. The fourth and sixth lobes which flank it on either side carry exceptionally large but otherwise normal cells, whereas the remainder conform to more orthodox proportions.

In the following pages the different species are taken up separately, the detailed analysis of *Mecistorhinus melanoleucus* being followed by briefer comparative accounts of the other forms. As far as possible, the interpretative treatment is relegated to the discussion that terminates the paper.

## Mecistorhinus melanoleucus

### *Normal lobes*

Except for certain features which are pertinent to an analysis of the peculiarities of the harlequin lobe, no detailed account of the spermatogenesis in the six normal

lobes need be given. It conforms closely in its general course to that which has often been described in other pentatomids.

The diploid set of fourteen chromosomes is marked by one exceptionally large pair of autosomes. This pair stands out almost as conspicuously as does the X chromosome of Protenor. Here however, the sex chromosomes are relatively small, the Y being the smallest member of the complement and the X little if any larger than the smallest of the autosomes (Fig. 2).

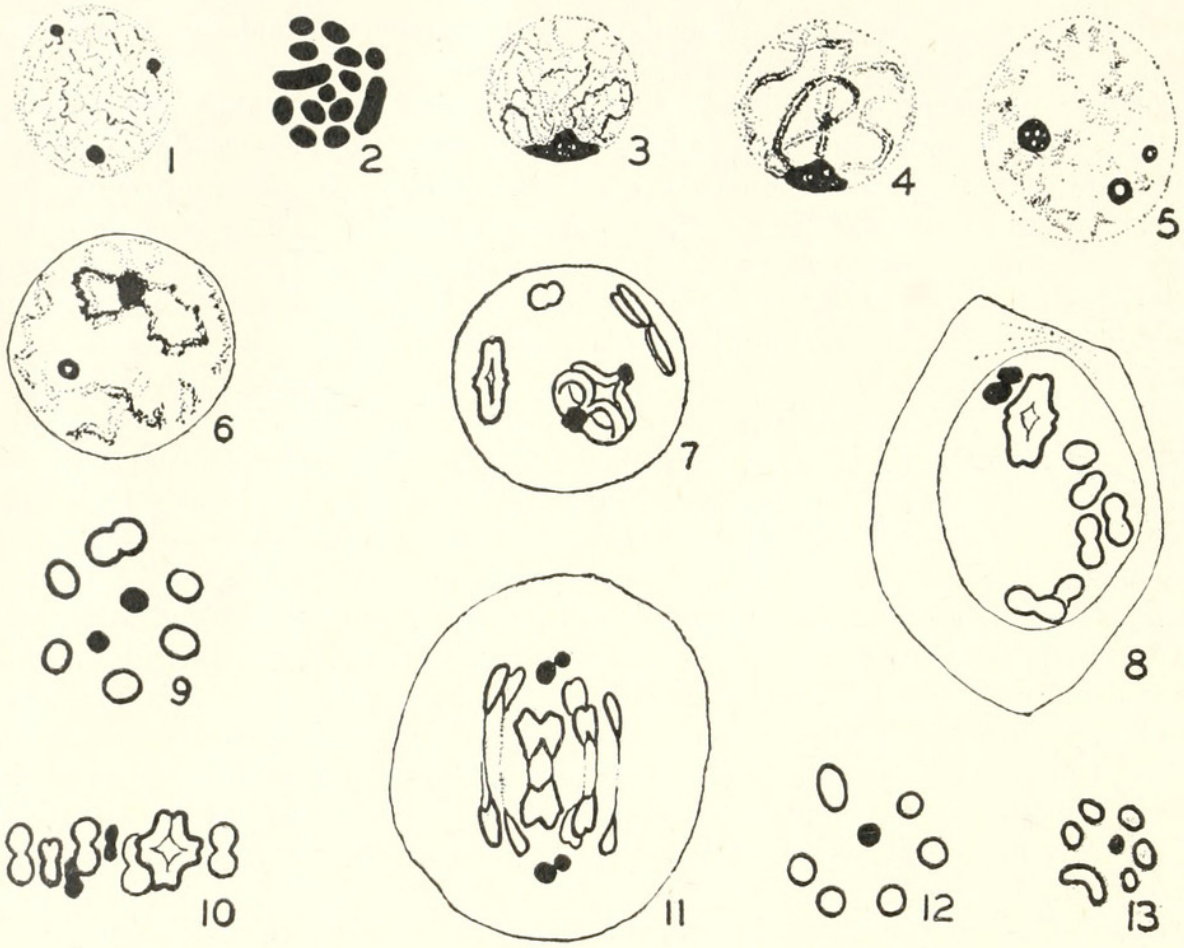
One of the exceptional features lies in the heteropycnosis that marks not only the sex chromosomes but also certain of the autosomes. Already in the early generations of the spermatogonia there are from three to five heteropycnotic bodies that stand out prominently in the resting phase (Fig. 1). There is no prochromosome stage intervening between the last spermatogonia and the leptotene stage of meiosis. In the leptotene and synaptic stages the heteropycnotic bodies are usually aggregated in a single mass on the nuclear periphery and it is at this locus also that the fine leptotene threads come together in a bouquet formation (Fig. 3). But in some cells there are two heteropycnotic bodies during these stages, the second and smaller one usually lying at some distance from the first and not necessarily at the periphery. The advent of the pachytene and diplotene stages sees little change (Fig. 4) in these conditions of heteropycnosis and it is only when they in turn give way to the confused stage that the single heteropycnotic aggregate is dissociated again. In this peculiar phase when staining conditions and despiralization temporarily convert most of the chromosomes into pale and flocculent threads there may again be three, four, or five heteropycnotic bodies (Fig. 5). This variation in number would seem to indicate that the mutual and nonspecific attraction that brings heteropycnotic chromosomal bodies together at certain stages is not very strong and it is likely that accidents of position determine these numbers to some extent.

In early diakinesis, as the chromosome threads again become definite in outline, the topographic relationships are once more open to analysis. Now the great majority of cells show only two heteropycnotic bodies, one of which becomes less and less conspicuous as the threads shorten and condense [1] while the larger one is seen to be intimately associated with the big bivalent (Fig. 6). Somewhat later, when the paired chromosomes have assumed the typical cross and ring formations of late diakinesis this large heteropycnotic body has disappeared, but there are then two smaller bodies, one associated with each of the two spreading arms of the large bivalent (Fig. 7). There is no doubt about the identity of these bodies. The larger is the X and the smaller the Y, the two together constituting the larger heteropycnotic body or chromosome nucleolus of earlier diakinesis. The dissociation of this single nucleolus into its two components is perhaps due not only to the strains that attend the separation of the arms of the large bivalent but may be a part of the regular cycle that in other species also sees the reappearance of the separate sex chromosomes at this stage. What is more remarkable is their persistent union with the arms of the autosomal bivalent, a union which is not broken until shortly before metaphase.

The rather even peripheral distribution of the diakinetic bivalents disappears

[1] The present state of our knowledge concerning the changes in the chromosome during a complete mitotic cycle is still unsatisfactory. Almost certainly both coiling and nucleination are involved, but the relative importance of these two factors remains undetermined. For that reason the terms "condensation" and "diffuseness" are here used in a purely descriptive sense.

just before the disintegration of the nuclear membrane. They then lie helter skelter in the nucleus and may even come into contact with each other. It is at this time when the chromosomes are in the final stages of condensation that the two sex chromosomes sever their connections with the large tetrad, though their former as-




*Mecistorhinus melanoleucus—Normal Lobe*

FIGURE 1. Early prophase in spermatogonial cell; three chromatin nucleoli (Feulgen).
FIGURE 2. Spermatogonial metaphase; Y is smallest of the 14 chromosomes (Feulgen).
FIGURE 3. Leptotene stage (Feulgen).
FIGURE 4. Diplotene stage (Feulgen).
FIGURE 5. Confused stage (Feulgen).
FIGURE 6. Early diakinesis; XY nucleolus attached to large tetrad (Feulgen).
FIGURE 7. Late diakinesis; X and Y attached to separate arms of large tetrad (Hematoxylin).
FIGURE 8. Prometaphase; X and Y still close to large tetrad (Hematoxylin).
FIGURE 9. Metaphase I; polar view (Hematoxylin).
FIGURE 10. Metaphase I; side view (Feulgen).
FIGURE 11. Anaphase I; large tetrad lagging (Gentian violet).
FIGURE 12. Metaphase II; polar view (Hematoxylin).
FIGURE 13. Telophase II; 6 autosomes + Y (Feulgen).

sociation is frequently indicated by their close proximity to it (Fig. 8). It is this stage also that is marked by an elongation of the nucleus as a whole in the polar axis, a change that plainly involves interaction with the two centers located at the periphery of the cell.

About the meiotic divisions themselves, little need be said. Metaphase I is quite typical in its conformation, with the now separated X and Y usually in the center of a ring of six tetrads (Fig. 9). It is however worthy of note that the large bivalent is somewhat slower than the rest of the autosomes in its condensation and side views of the first equatorial plate still show it as a cross tetrad (Fig. 10). At anaphase the X and Y divide equationally and arrive at the poles before the rest of the chromosomes, whereas the large bivalent (often showing the tertiary split) lags in its division and is distinctly slower than the other autosomes in its anaphasic progress (Fig. 11).

The second division also witnesses some lagging on part of the large autosome, but this is not as striking as in the first division. The touch and go pairing of the X and Y occurs as usual, and in metaphase they line up in the spindle axis so that in polar views one is superimposed on the other (Fig. 12). They then separate to oposite poles and the spermatids receive the typical pentatomid complements of 6A + X and 6A + Y respectively (Fig. 13). The departures from the orthodox process of meiosis thus do not affect the results, which conform to the regular pentatomid scheme.

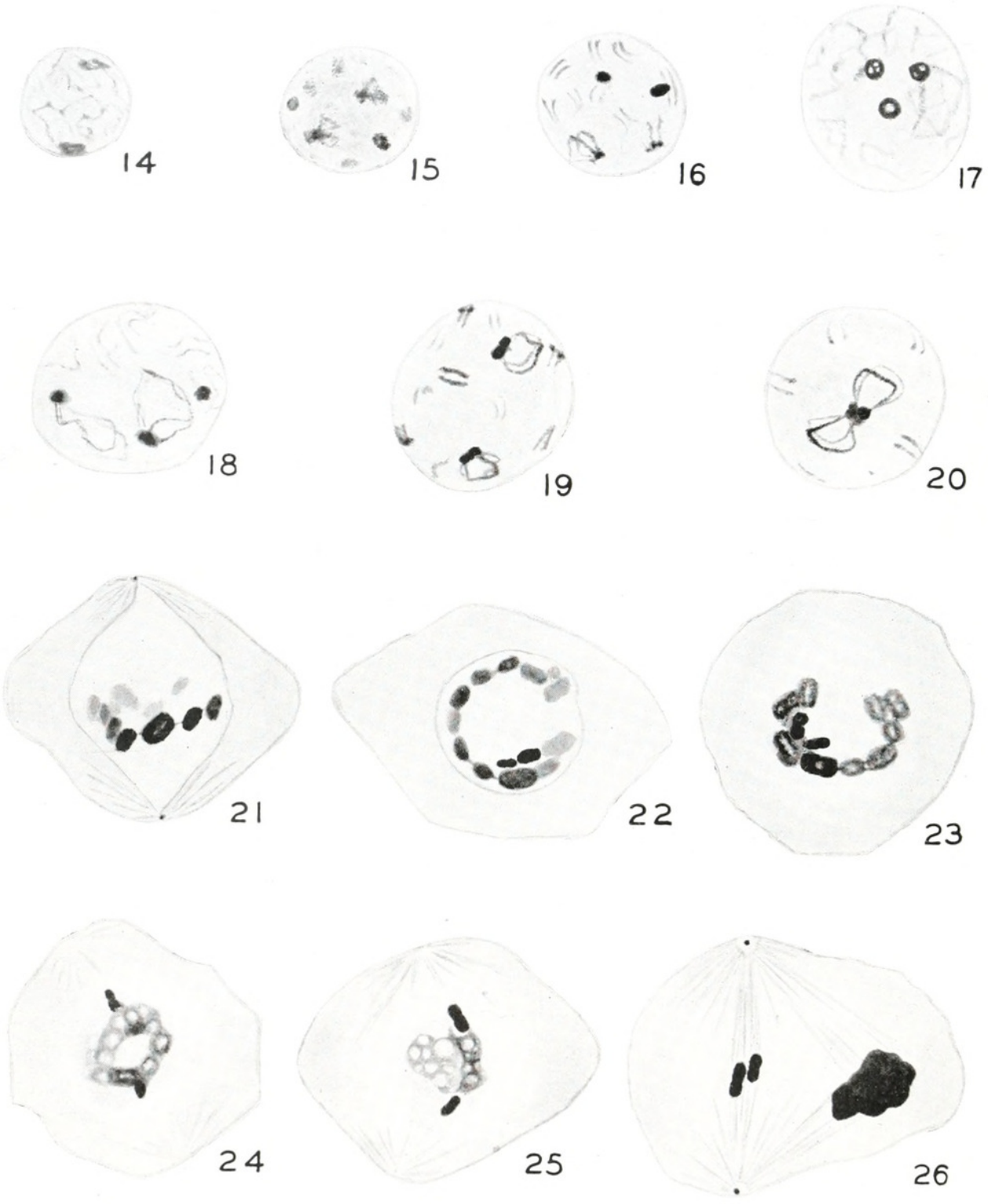
*Harlequin lobe*

### Spermatogonia and meiotic prophases

The spermatogonial stages in the harlequin lobe differ in no discernible way from those of the normal lobes. Here too there are, in Feulgen preparations, from three to five heteropycnotic bodies in the resting phase and the succeeding stages closely parallel the normal course of events. As in the normal lobes there is no prochromosome stage. The meiotic leptotene duplicates that of the other lobes in the number and disposition of heteropycnotic bodies, but the chromosome threads do not seem to be as finely drawn out and delicate as they normally are. This difference however is too slight to furnish a secure basis for contrast (Fig. 14). Succeeding this stage the developments follow a path that diverges widely from the usual one.

There is neither a synapsis nor a pachytene stage. During the period in which these developments occur in the normal lobes, the chromosome threads of the harlequin lobe merely abandon their bouquet orientation and undergo a progressive condensation. As a result the nucleus then shows twelve somewhat loosely coiled autosomes and the two more condensed sex chromosomes, clear evidence that any sort of pairing that may have occurred unobserved prior to this time has now been abrogated (Fig. 15). The picture presented is a surprisingly close approximation of the prochromosome stage as it occurs normally in some Hemiptera, and in this respect has some resemblance to the conditions in the harlequin lobe of Loxa (Schrader, 1945b) where such a post-leptotene condensation is also encountered. But in Loxa there is a true prochromosome stage as well which occurs quite normally prior to the evolution of the leptotene threads. Both there and in Mecistorhinus no confusion is possible for not only does the true prochromosome stage occur much higher in the testis, but its nuclei are considerably smaller than are the ones here in question.

This post-leptotene condensation culminates in shortened, fuzzy chromosomes that show an equational split (Fig. 16), undoubtedly a condition corresponding to




*Mecistorhinus melanoleucus—Harlequin Lobe*

FIGURE 14. Leptotene stage (Gentian violet).
FIGURE 15. Post leptotene condensation; X and Y heteropycnotic (Feulgen).
FIGURE 16. Diplotene stage in univalents (Gentian violet).
FIGURE 17. Late confused stage (Feulgen).
FIGURE 18. Early diakinesis; sex chromosomes attached to separate large autosome (Gentian violet).
FIGURE 19. Mid-diakinesis; 12 univalents autosomes, with each large autosome combined with one sex chromosome (Gentian violet).

the diplotene stage of the normal lobes. Throughout this period two pairs of chromosomes are readily recognizable; they are the two large autosomes and the two heteropycnotic sex chromosomes. Each large autosome has its ends united so as to form a split ring, a configuration that very probably arises from the mutual attraction of its heteropycnotic terminal regions. The two heteropycnotic sex chromosomes show no such attraction at this time and usually lie well separated, evidence that heteropycnotic attraction is confined to certain conditions of the heterochromatin.

The confused stage which now intervenes, temporarily halts a further close analysis of progressive chromosome changes. The autosomes once more become diffuse and uncoiled and at the height of the stage stain very lightly. Usually three heteropycnotic bodies are present at this time, but there may be as many as five (Fig. 17). In the latter case the bodies are smaller, generally speaking, which would indicate that the variations in number are due to some vagaries in mutual attraction and aggregation. The two sex chromosomes and the ends of the two autosomes would account for six such bodies which suggests that some aggregation is nearly always present.

With the termination of the confused stage and the beginning of diakinesis, the individual chromosomes once more appear as such. There are then three heteropycnotic bodies and two of these are seen to be associated with the two large autosomes (Fig. 18). The third shows no such definite association and gradually disappears. In mid-diakinesis a more exact analysis of these conditions becomes possible. At this time there is a total of either eleven or twelve chromosomal bodies in every nucleus. When there are twelve, the two autosomes are quite independent of each other and may lie far apart. Each of them has a large chromatin nucleolus or heteropycnotic body attached to it at the place where the ends are still joined in ring formation (Fig. 19). When on the other hand there are only eleven bodies, these two chromatin nucleoli have come together, and through them the two large, ring formed autosomes have joined in a figure eight (Fig. 20). The two chromatin nucleoli represent the X and Y chromosomes and again, the most natural explanation of such configurations would seem to lie in the forces of heteropycnotic attraction; the heteropycnotic ends of the large autosomes are drawn together to form rings, and the heteropycnotic sex chromosomes later become attached to these regions and to each other for the same reason. It is rather strange that no case has been encountered in which both sex chromosomes have become joined to only one of the large autosomes, since nonspecific heteropycnotic attraction might be expected to give rise occasionally to such configurations. However, nuclei of this stage in which the chromosomes are open to a clear analysis are not common and

FIGURE 20. Mid-diakinesis; both sex chromosomes and both large autosomes in one combination (Gentian violet).

FIGURE 21. Equatorial ring side view (Hematoxylin).

FIGURE 22. Equatorial ring slightly later; polar view (Gentian violet).

FIGURE 23. Autosomes in precocious return to diffuse condition; X and Y still heteropycnotic (Gentian violet).

FIGURE 24. Formation of autosomal aggregate; X and Y heteropycnotic (Gentian violet).

FIGURE 25. Dissociation of X and Y from autosomal aggregate (Gentian violet).

FIGURE 26. Metaphase I; X and Y on middle spindle and autosomal aggregate displaced (Hematoxylin).

the fourteen examples which have been studied hardly constitute a sufficiently large number to justify the conclusion that they do not occur.

### Prometaphase

Shortly before the breakdown of the nuclear membrane a significant reorientation of the chromosomes takes place. This is at about the time that the nucleus elongates toward the peripherally located centers. The chromosomes, still not fully condensed, then are shifted to the middle region between the centers and since they remain in close proximity to the nuclear wall and have lost the property of mutual repulsion, they tend to form a more or less circular row or chain in the equator. Some of the components of such chains may be in actual contact with each other, while others may be connected by Feulgen positive bridges or show no attachment at all (Figs. 21, 22, and 72). It is likely that such bridges are similar in nature to those seen later at metaphase (see for instance Ris, 1942), but whether they represent viscous connections that persist after a former contact or are indicative of a "reaching out" of chromosomes toward each other, it is impossible to decide.

When the nuclear membrane finally disappears, this picture undergoes marked and sudden changes. The chain of chromosomes, now free of the influence of the membrane, seems to collapse inwardly, frequently forming a closed ring at first and then an irregular aggregate in the middle of the nuclear space. In the many cells seen at this and the following stages no instance of more than a single aggregate has ever been observed, a point of difference with the case of Brachystethus (Schrader, 1946). Concurrently with these changes of orientation there occur alterations in the chromosomal structure. These are marked especially by a partial return to the diffuse condition in the autosomes, with an accentuation of the equational split. The two large autosomes do not seem to become quite as diffuse as the rest, but this difference is not a striking one at best. This return to a more diffuse state causes the autosomal aggregate to appear as a spongy and vacuolated mass in both gentian violet and Feulgen preparations and this condition is maintained for the major part of the first division (Fig. 23). Hematoxylin slides allow no such structural diagnosis for there the aggregate is nearly always homogeneously and intensely stained.

The behavior of the sex chromosomes is remarkable during the prometaphase and the establishment of the metaphase itself. At the time of the equatorial ring formation, just prior to the disintegration of the nuclear membrane, they are still very close or even in contact with the large autosomes. Almost always they lie on the inner side of the ring and not in seriation with the rest of the chromosomes (Fig. 22). They seem to be almost fully condensed at this time and are recognizable in most cells. When the autosomal chain collapses to form the irregular aggregate, this distinction becomes even more marked, for in contrast to the autosomes they then maintain their condensed state and in addition tend to protrude from the spongy mass of autosomes (Fig. 24). This protrusion seems to be an indication of interaction with the two centers, for the sex chromosomes not only make their appearance on the side toward one of the poles but begin to place their long axis in alignment with the polar axis (Fig. 25) which is a placement assumed also at the ensuing metaphase.

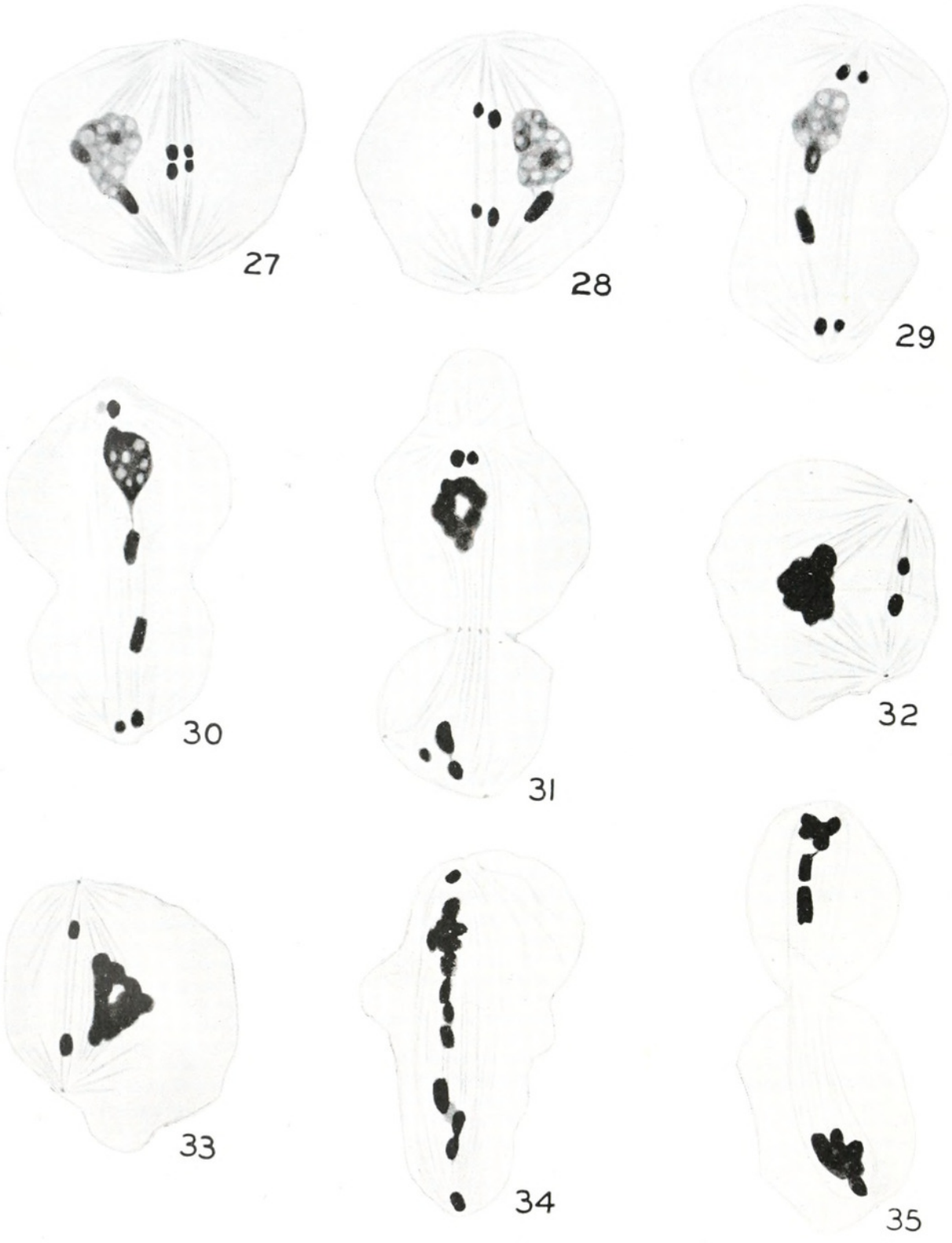
### The first division

The clumping of the partially diffused autosomes brings about a rather anomalous situation and the establishment of the metaphase can be followed only through the behavior of the sex chromosomes. These appear to be quite normal in their further maneuvers. They finally become completely detached from the spongy aggregate of autosomes and take up an equatorial position side by side. During this movement several other developments occur simultaneously. Chromosomal fibers appear connecting the sex chromosomes as well as the aggregate with the poles, and at the same time the whole mass of autosomes is shunted out of the middle region toward the side of the cell. This shift must be rather sudden, for intermediate stages are very rare. In extreme instances the displacement may bring the autosomal aggregate very close to the side of the cell, though never touching it, and in every case it comes to lie farther from the polar axis than from the cell wall. While in this position, two points are to be noted: the autosomal aggregate remains connected with the poles through definite chromosomal fibers, and even in its displacement it maintains an equal distance from both poles (Figs. 26, 73, and 74). The whole reaction is obviously closely akin to a similar one observed in the pentatomid Brachystethus (Schrader, 1946).

The two sex chromosomes apparently are not affected by the anomalous behavior of the autosomes. They lie side by side in a compact and narrow spindle of normal length and undergo an orthodox equational division. In some cells the chromatids of the Y separate faster than those of the X and may precede them to the poles. As soon as the anaphase movement of the sex chromosomes is initiated, the autosomal aggregate once more approaches the polar axis, and by mid-anaphase is usually close to or even in contact with the sex chromosome spindle. This return also occurs in the equatorial plane of the cell, and is correlated with a shortening of the chromosomal fibers as well as the lengthening of the interpolar distance and cell as a whole—both of which will of course bring the autosomal aggregate closer to the polar axis again (Figs. 27 to 29).

Although the autosomes, aggregated as they are, pass through these maneuvers as a unit, there is evidence from the beginning that one of them plays a special role. Already at the first trace of division in the X and Y chromosomes, a single large chromosome protrudes from the autosomal clump, showing a well formed chromosomal fiber connection with one center and clearly oriented toward it. In such a position it appears more condensed than the rest of the autosomes, a condition which would be difficult to discern while it is still in the midst of the vacuolated, unevenly staining aggregate (Figs. 27 and 28). The reaction of this autosome to the pole is quite independent of the sex chromosomes, but it is obviously hindered in its movements—probably because of the "stickiness" that tends to hold all the autosomes together. As a result the two sex chromosomes are well on their way toward the poles before this autosome has disengaged itself from the encumbrance (Figs. 29 and 75).

Soon after it has left the aggregate, a second large autosome begins to dissociate itself from the rest of the autosomes. The extent to which it succeeds in this is highly variable in different cells, but in most cases it at least protrudes from the mass before the division is finished (Fig. 30). Often, while the first autosome is still fairly




*Mecistorhinus melanoleucus—Harlequin Lobe*

FIGURE 27. Early anaphase I; autosomal aggregate beginning to return (Gentian violet).
FIGURE 28. Mid-anaphase I; (Gentian violet).
FIGURE 29. Late anaphase I (Gentian violet).
FIGURE 30. Early telophase I (Gentian violet).
FIGURE 31. Late telophase I (Hematoxylin).
FIGURE 32. Early anaphase II—large cell (Gentian violet).
FIGURE 33. Mid-anaphase II—large cell (Hematoxylin).
FIGURE 34. Early telophase II—large cell (Hematoxylin).
FIGURE 35. Telophase II—large cell (Hematoxylin).

close to the aggregate, it shows a Feulgen-positive connecting thread with the second one, although this is usually severed very shortly (Fig. 29).

The rest of the autosomal clump, which is by now very close to the polar axis, also shows some response to the mitotic forces and frequently undergoes some elongation in the polar direction. Its effective movement however is always opposite in direction to that of the first autosome and hence it approaches the other pole. The second large autosome may sometimes almost reach the middle of the cell, but in only a single case has it proceeded so far that the ensuing cleavage constriction promises to include it in the same cell with the first autosome. Indeed, a count of 100 small second spermatocytes (those which do not receive the main part of the autosomal aggregate) has revealed no such accidental inclusion and it must be very rare. Further, in all observed cases (of which there are many dozens) this second autosome has rejoined the aggregate by the time that the second division is begun so that its mitotic motion must finally be reversed (Fig. 31). Possibly it initially follows the large autosome only because it is dragged along by the connecting thread. The final result of these maneuvers is that the large autosome and the rest of the autosomal aggregate always go to opposite poles. Since the former is the first to evince any reaction to the mitotic forces, one is almost forced to the hypothesis that it determines the direction of movement on the part of all the remaining autosomes.

These two autosomes that tend to disengage themselves from the aggregate are patently larger than any but the two largest chromosomes of the diploid set. At the same time they do not seem quite to reach the size of that large pair and it may therefore be that we are dealing with the two chromatids of only one of the latter. But admittedly the changes in the state of autosomal condensation during meiosis make it difficult to decide the matter on the basis of size alone. The later behavior of these exceptional chromosomes in the spermatids would however seem to support their identification as chromatids rather than whole chromosomes.

The second spermatocytes resulting from this first division, anomalous though it may be, are thus very constant in composition. Half of them contain the two sex chromosomes and only one autosome; the rest carry all the remaining autosomes as well as the sex chromosomes. The latter are much the larger cells of the two, as might be expected (Fig. 31).

### The second division

*Large cell:* The chromosomes carried by these second spermatocytes comprise the sex chromosomes as well as all of the autosomes except the single one in the smaller cell. There is no interkinesis. In its general aspects the division of this cell simulates the first division. As the X and Y take their position on the new spindle, chromosomal fibers are also formed between the two poles and the autosomal aggregate. Almost simultaneously, the latter is shifted toward the side of the cell, the displacement being very similar to that observed in the first division (Figs. 32 and 76). However the aggregate now begins to be less spongy in appearance and there are other indications that its autosomal constituents are once more undergoing condensation (Fig. 33). During the anaphasic separation of the X and Y, the aggregate is again drawn toward the middle of the cell. It may be noted that when it reaches the compact little sex chromosome spindle, its surface of contact with it becomes smooth and concave, indicating that it is under some pressure (Fig. 33).


*Mecistorhinus melanoleucus—Harlequin Lobe* (except Figs. 41 and 43)

FIGURE 36. Beginning of Division II—small cell; large autosome connected with both X and Y (Hematoxylin).
FIGURE 37. Early anaphase II—small cell (Hematoxylin).
FIGURE 38. Late anaphase II—small cell (Hematoxylin).
FIGURE 39. Early telophase II—small cell (Hematoxylin).
FIGURE 40. Late telophase II—(Hematoxylin).
FIGURE 41. Early spermatids—normal lobe (Hematoxylin).
FIGURE 42. Early spermatids—harlequin lobe (Hematoxylin).
FIGURE 43. Late spermatids—normal lobe (Hematoxylin).
FIGURE 44. Late spermatids—harlequin lobe (Hematoxylin).

Then as the anaphase progresses and the cell and spindle elongate, the aggregate is drawn out between the two poles and many of its component autosomes are thus strung out in a roughly linear order (Figs. 34 and 78). But the tendency to stick and adhere to each other remains strong so that even when several individual chromosomes have been pulled out of the aggregate they usually still show thick, Feulgen-positive connections with each other and with the remaining aggregate (Figs. 34 and 35). The division so far as the autosomes are concerned is therefore obviously a haphazard one and the aggregate is frequently distributed very unevenly to the two spermatid cells.

The spermatids resulting from the division of the large second spermatocyte are therefore exceedingly variable in composition. Since the sex chromosomes segregate normally, all spermatids carry either an X or a Y, but the number of autosomes is largely a matter of chance. Nevertheless no cases have been observed in which the latter have all gone to one pole.

*Small cell:* As is the case with the large second spermatocytes there is no interkinesis of any kind and the final stages of the first division merge directly into the beginnings of the second. Indeed before the anaphase movement of the first division has been completed, the two daughter centrioles at each pole (each centriole appears double already at metaphase) have separated and begin their migration to establish the polar axis of the second division, at right angles to the first (Fig. 31). In some cells the sex chromosomes respond and orient to these new poles before they have completed the anaphasic movement. But apparently this precocious movement is then reversed, for in slightly later phases when the new axis has been established, the two sex chromosomes are near each other or in actual contact in the middle of the cell. Here too now lies the large autosome which has been delayed in its arrival at the pole. It may be in contact with neither, either or both sex chromosomes, or it may be connected with either or both through Feulgen-positive bridges (Fig. 36).

Chromosomal fibers are formed already before the three chromosomes have gathered at the midpoint, but so far as can be seen the tiny spindle is concerned only with the X and Y. No chromosomal fibers can with certainty be traced to the autosome. The sex chromosomes behave just as they do in a normal cell and after meeting in the middle of the spindle they separate to opposite poles in a regular segregation.

It is the behavior of the autosome that presents some puzzling aspects, as it already has done in the first division. The outstanding feature of this behavior lies in the fact that it nearly always goes to the same pole with the X. In so doing it appears to have little or no independent mitotic movement, acting merely as a satellite of the sex chromosome. Its dependence on the latter is shown not only by the absence or poor development of chromosomal fibers already mentioned, but is indicated also by its behavior before and during anaphase. In the grouping prior to the division, the autosome sometimes lies between the X and the adjacent pole, but when the anaphase movement is under way it always trails the sex chromosome (Figs. 36 to 40, and 77). Since during most late anaphases the autosome is attached more or less closely to the X, its behavior might be attributed to a simple adhesion between the two were it not for the fact that many earlier anaphases show no such connection. The latter thus frequently seem to be established after the mitotic movement is under way. Indeed, connections of this sort cannot be decisive in any case since prior to the division the autosome often lies in contact with both

sex chromosome or shows a Feulgen-positive bridge to the Y and not to the X (Fig. 36). Evidently this is later broken so that the mitotic association with the X must involve some selective action not dependent on such physical bonds. Whatever the underlying mechanism may be, the results of the division admit of no doubts concerning the constancy of the relationship between these two chromosomes. In 100 clear side views of late anaphases there were only six that admitted the possibility of an association of the autosome with the Y. In two of these the autosome is clearly much closer to the Y than the X and probably going to the same pole, whereas in the remainder the identification of the X and Y is not certain. In close to 95 per cent of the cases therefore, the autosome accompanies the X to the pole and not the Y. Generally speaking then, the small spermatids which come from this division are of two types: one, which carries the X and the autosome; and another which contains only the tiny Y.

### Spermatids and sperms

The two anomalous spermatocyte divisions of the harlequin lobe thus give rise to four main types of spermatids: X + one large autosome; Y; X + a variable number of autosomes; Y + a variable number of autosomes. Since in the spermatid the autosomes scatter again and tend to be distributed peripherally on the new nuclear membrane before becoming diffuse, rather dependable counts are often possible. Both of the small types of spermatids are readily recognizable and it is to be noted that the close association between the X and the autosome of the preceding telophase is maintained into an advanced spermatid stage (Fig. 42). In the large spermatids the number of chromosomes may be as low as five or six and frequently higher than twenty. The latter counts constitute conclusive evidence that the chromatids of the univalent autosomes have separated from each other, since the full, normal haploid number of the species is only seven (compare the normal spermatids of Fig. 41 with those of the harlequin lobe in Fig. 42). Since the autosome that is associated with an X in one of the small spermatids undergoes no such separation into smaller units, there is strong presumptive evidence (to support that which was adduced earlier) that it represents a chromatid which has already separated from its sister chromatid—the second large autosome of the first division.

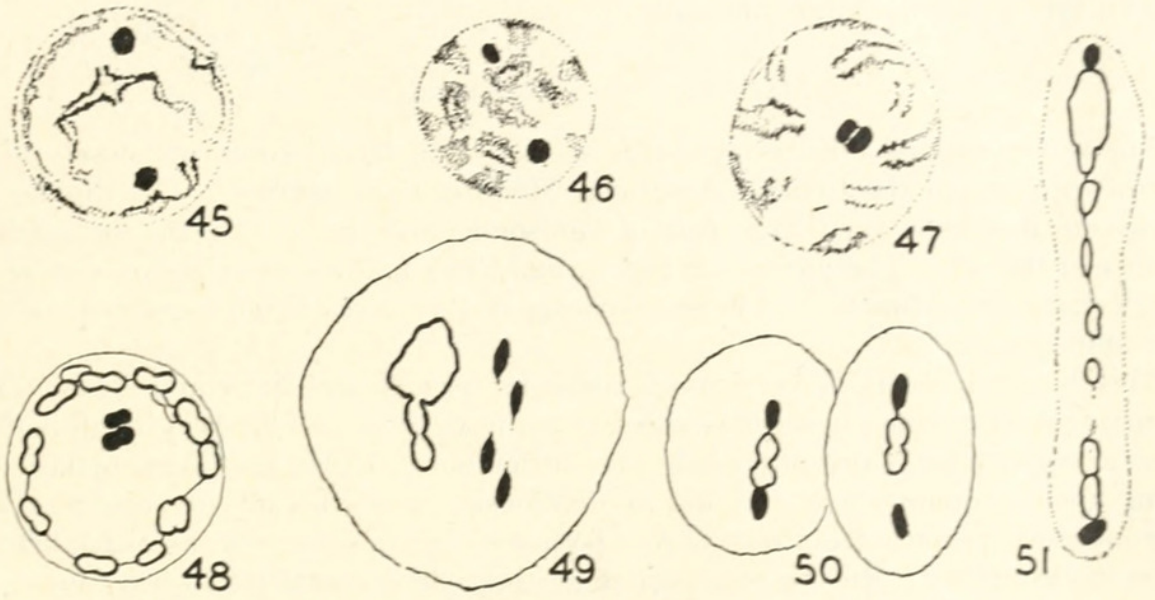
Formation of sperms seems to proceed normally in all cells until the stage when the elongation nucleus shows the pointed apex which indicates the presence of the acrosome (compare Fig. 43 with Fig. 44). At about this time, when the chromosomes have become very diffuse and the contents of the sperm head seem structureless, the small types of sperms gradually stain more and more faintly. It finally becomes impossible to recognize them, whereas the larger types assume the attenuated, intensely stained form of the head that is also encountered in the normal lobes. It is more than probable therefore that the smaller sperms never attain maturity, whereas the large ones appear normal in every respect but size. They enter the sperm duct in a perfectly regular manner and there mingle with the sperms of the normal lobes.

## Mecistorhinus tripterus

The main points in which *Mecistorhinus tripterus* differs from *Mecistorhinus melanoleucus* involve the heteropycnosis of the autosomes and the behavior of the

sex chromosomes. So far as the diploid complement of chromosomes is concerned, there is no perceptible difference, both having an outstandingly large pair of autosomes and a Y that is the smallest of all the chromosomes. However, already in the spermatogonial resting phases there is a less extensive heteropycnosis in *Mecistorhinus tripterus*, for then as well as later there are never more than two heteropycnotic bodies (instead of from three to five). The spermatogenesis in the normal lobes is entirely orthodox. The heteropycnosis in the large autosomes is here lacking entirely and hence there is no association between them and the sex chromosomes (Fig. 45).

The lack of autosomal heteropycnosis obtains also in the harlequin lobe (Figs. 46 and 47). As a consequence, when the equatorial ring is formed the two sex




*Mecistorhinus tripterus—Harlequin Lobe* (except Fig. 45)

FIGURE 45. Normal diakinesis; X and Y independent of autosomes (Feulgen).
FIGURE 46. Early diplotene stage (Feulgen).
FIGURE 47. Diakinesis; X and Y joined (Feulgen).
FIGURE 48. Equatorial ring in formation; X and Y independent of autosomes (Feulgen).
FIGURE 49. Anaphase I (Feulgen).
FIGURE 50. Anaphase II—small cell (Feulgen).
FIGURE 51. Anaphase II—large cell (Feulgen).

chromosomes lie in the approximate middle of the nuclear space instead of being carried to the periphery by the large autosomes (Fig. 48). The X and Y are at this time still in more or less intimate contact with each other and remain so until the first anaphase has begun.

Another difference lies in the size relations between the X and the Y. In *Mecistorhinus melanoleucus* the X is markedly larger than the Y, but this difference is less pronounced in *Mecistorhinus tripterus*. In the latter also, as in most other pentatomids, the sex chromosomes become less sharply outlined in the second division and therefore it then becomes difficult at times to distinguish between them (Fig. 50). As a consequence, in about 25 per cent of fifty cells it is not possible to decide whether the autosome follows the X and there is at least the possibility that in such

instances it accompanies the Y. Although there is thus no question that in this race too the association is between the X and the autosome in the great majority of cells, the case is not as clear-cut as it is in *Mecistorhinus melanoleucus*.

Mention should also be made of the fact that already in the first division, the first large autosome usually shows a median furrow (Fig. 49). If, as in *Mecistorhinus melanoleucus* this chromosome represents only one chromatid of one of the large univalents, the furrow is equivalent to a tertiary split. Whether that be correct or not, this split is not consummated even in the early spermatid, where the large autosome still maintains this appearance.

But in essence, the meiotic divisions of the two species are very much alike (Figs. 49–51, and 78). The difference in heteropycnosis does not affect the results and *Mecistorhinus tripterus* merely furnishes less decisive evidence anent the association between the X and the large autosome.

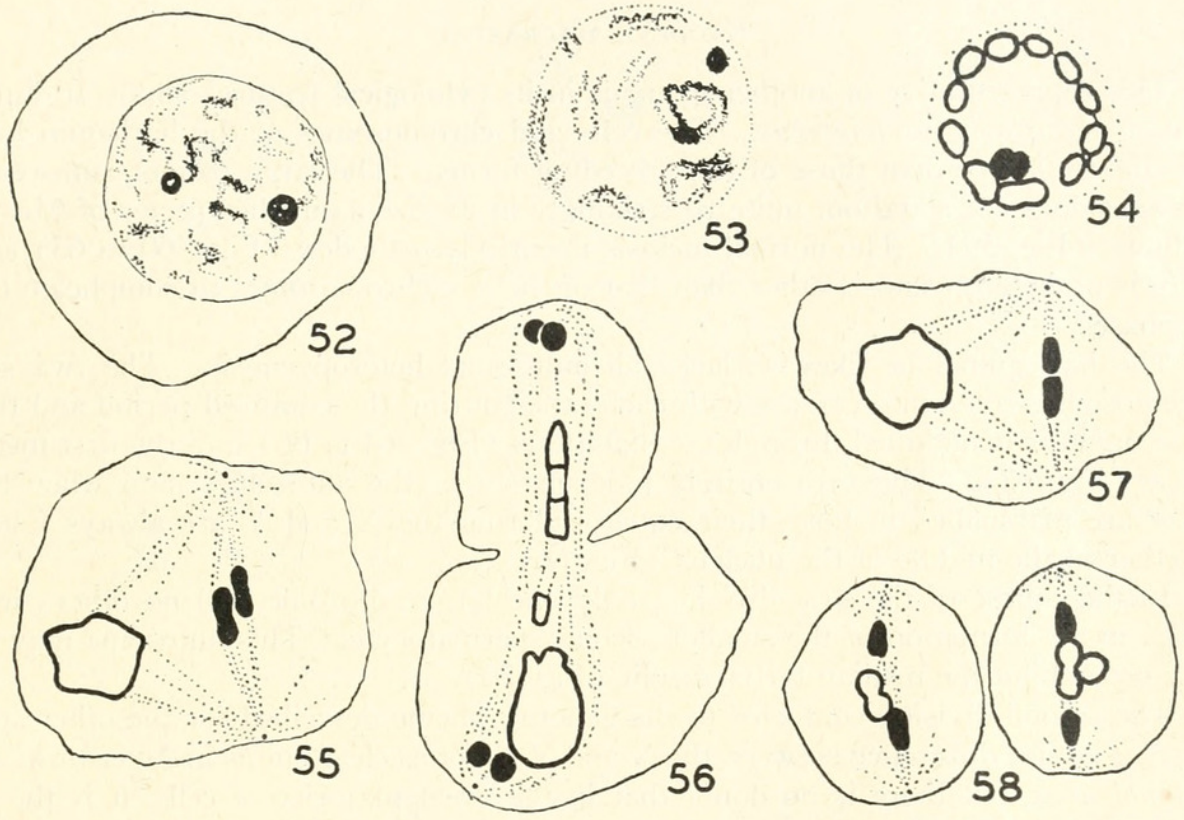
### Mecistorhinus sepulcralis

The rather extensive material of this species from Brazil conforms closely in its general cytology to the Central American Mecistorhinus species. The diploid set is characterized by a very large pair of autosomes and the Y is again the smallest member of the set. The meiosis in the normal lobes follows an orthodox course although there are indications of heteropycnosis in a very restricted region of the two large autosomes.

This heteropycnosis is very much alike in normal and harlequin lobes. The spermatogonial resting phases may show as many as three or four very small heteropycnotic regions but more often only one larger one. In the meiotic prophase following the leptotene stage and up to diakinesis, it is difficult to recognize any heteropycnosis in the autosomes whereas both sex chromosomes are, as usual, heteropycnotic throughout. In the confused stage there are generally two heteropycnotic bodies, but whether these represent only the sex chromosomes or also certain autosomal regions it is not possible to say (Fig. 52). Only in mid-diakinesis do conditions allow a closer analysis. In the harlequin lobe, where we are dealing with univalents, both large autosomes then show heteropycnotic terminal regions, and these are joined so as to form closed rings as in *Mecistorhinus melanoleucus* (Fig. 53). Apparently this heteropycnosis is less extensive in the present species and this may account for the fact that the mutual attraction of such regions does not bring the two large univalents together in the "figure eight" formations that occur so often in the other forms. Probably for the same reason the association between the sex chromosomes and these autosomes is highly variable. Thus in some cells only one sex chromosome is joined to the heteropycnotic region of one univalent autosome while the other sex chromosome is free (Fig. 53). In late diakinesis such a condition is rare because, as in *Mecistorhinus tripterus,* there is then a strong tendency for the X and Y to come together. Hence the free sex chromosome often joins its attached mate and as a result both are carried into the equatorial ring of autosomes, just prior to the breakdown of the nuclear membrane (Fig. 54). In other cases the sex chromosomes may be entirely free and will then take a more or less central position in the nucleus at the time of the equatorial orientation of the autosomes.

But these various maneuvers that involve heteropycnotic attraction do not affect the course of the actual divisions in the harlequin lobe any more than in the normal

lobes. In the former the sex chromosomes at both metaphases become detached from the autosomal aggregate, which then is displaced toward the side of the cell just as in *Mecistorhinus tripterus* (Figs. 55 and 57). In *Mecistorhinus sepulcralis* however, the first anaphase shows a definitely greater tendency for other chromosomes to follow the first large autosome out of the aggregate. Although the second large autosome is included as rarely in the smaller second spermatocyte as in the preceding species, a smaller autosome sometimes succeeds in following the first autosome into this cell (in four out of forty cells) (Fig. 56). Apparently both these autosomes




*Mecistorhinus sepulcralis—Harlequin Lobe*

FIGURE 52. Confused stage (Feulgen).
FIGURE 53. Diakinesis stage (Feulgen).
FIGURE 54. Equatorial ring; X and Y associated with large autosomes (Feulgen).
FIGURE 55. Metaphase I (Hematoxylin).
FIGURE 56. Late anaphase I (Hematoxylin).
FIGURE 57. Metaphase II—large cell (Hematoxylin).
FIGURE 58. Anaphase II—small cell (Hematoxylin).

retain the "sticky" condition of the earlier aggregate, for when they reach the pole the smaller joins the larger one and adheres to it in almost any position in random fashion. In the second division, both follow the X to the pole in the majority of cases (Fig. 58).

But here too, as in the second division of *Mecistorhinus tripterus* the X and Y often show only a small size difference In perfect side views they can nearly always be distinguished, but if the cell is viewed at a slight angle it at once becomes difficult to do so. Hence, though *Mecistorhinus sepulcralis* also shows a definite preferential association between the X and the large autosome (as well as the smaller, if present)

the possible occurrence of exceptions to the rule can no more be excluded than in the preceding form.

The division of the aggregate in the larger second spermatocyte occurs very much as it does in *Mecistorhinus tripterus*. The four main types of spermatids are therefore alike in all three species, comprising X + one autosome; Y; X + variable number of autosomes; Y + variable number of autosomes. The few exceptions in the present species are due to the occasional inclusion of a smaller autosome in the first named spermatid.

### Neodine macraspis

This representative of another genus is in its cytological features almost a duplicate of *Mecistorhinus tripterus*. The cells and chromosomes of the harlequin lobe are slightly larger than those of the preceding forms. The large pair of autosomes however does not stand out quite so strikingly in its size as in the species of Mecistorhinus (Fig. 59). The normal meiosis is entirely orthodox (Figs. 60 to 63) and there is no heteropycnosis other than that of the sex chromosomes to complicate the prophases.

The harlequin lobe likewise lacks all autosomal heteropycnosis. The two sex chromosomes come into contact with each other during the confused period and this loose union is maintained through the diakinesis (Figs. 64 to 66) into the first metaphase (Fig. 67). They are entirely independent of the autosomes, and when the latter are marshalled to form their equatorial ring the X and Y are always found together in the middle of the nucleus (Fig. 66).

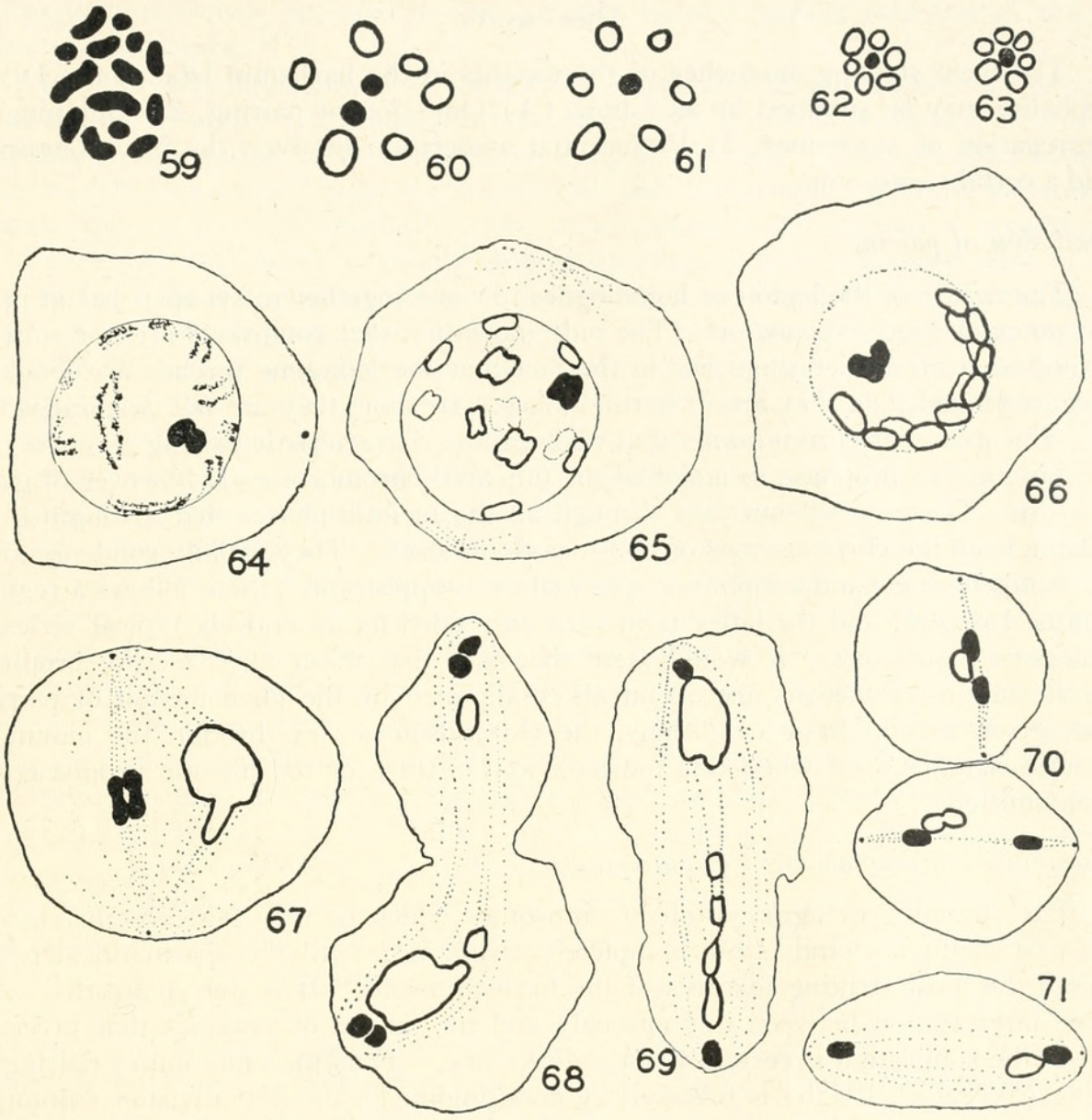
In the course of the first division, only one large autosome and no others join the X in the formation of the smaller second spermatocyte. This autosome may or may not exhibit the median tertiary split (Fig. 70).

The second division conforms to the general scheme described for the other species. The size difference between the X and Y is not as clear cut as in *Mecistorhinus melanoleucus*, but there is no doubt that in the great majority of cells, it is the X with which the single autosome is associated and not the Y (Figs. 70 and 71). The larger second spermatocyte divides irregularly and in the same manner as does the corresponding cell in Mecistorhinus.

In all essentials, therefore the meiosis in the harlequin lobe of Neodine parallels that of Mecistorhinus.

### Platycarenus notulatus

In view of the uniformity in the occurrence of normal and harlequin lobes of the four preceding species, it comes somewhat as a surprise that in another species, *Platycarenus notulatus*, the conditions are entirely different. The structure of the testis departs radically from that of the other forms, there being only four instead of seven lobes, with a more compact instead of a serial arrangement. The diploid set of fourteen chromosomes is marked by no strikingly large pair of autosomes and only the exceptionally small size of the Y merits any notice. There is no harlequin lobe and the spermatogenesis takes a very orthodox course throughout the testis. The case thus serves warning that the cytological conditions are by no means uniform in the tribe Discocephalini as it is now constituted by the systematists.




*Neodine macraspis*

*Normal Lobe:*

FIGURE 59. Spermatogonial metaphase (Hematoxylin).
FIGURE 60. Metaphase I (Hematoxylin).
FIGURE 61. Metaphase II (Hematoxylin).
FIGURE 62. Telophase II; polar view with Y (Hematoxylin).
FIGURE 63. Telophase II; polar view with X (Hematoxylin).

*Harlequin Lobe:*

FIGURE 64. Early diakinesis; joined X and Y independent of autosomes (Hematoxylin).
FIGURE 65. Late diakinesis; 12 univalents + joined XY (Hematoxylin).
FIGURE 66. Formation of aggregate; joined X and Y separate (Hematoxylin).
FIGURE 67. Early anaphase I (Hematoxylin).
FIGURE 68. Telophase I (Hematoxylin).
FIGURE 69. Telophase II—large cell (Hematoxylin).
FIGURE 70. Anaphase II—small cell (Hematoxylin).
FIGURE 71. Telophase II—small cell (Hematoxylin).

## Discussion

The most striking anomalies of the meiosis in the harlequin lobe of the Discocephalini may be summed up as follows: 1) Omission of pairing, 2) Clumping or aggregation of autosomes, 3) Preferential association between the X chromosome and a certain autosome.

### *Omission of pairing*

The failure of the leptotene homologues to come together in synapsis has at present no cytological explanation. The only indication that conditions are not entirely orthodox at an earlier stage lies in the fact that the leptotene threads are less fine and attenuated than they are in normal lobes. In short they are not despiralized to the same extent as chromosomes that undergo the characteristic pairing reaction.

The further prophase behavior of the unpaired chromosomes is however of great interest. These univalents pass through all the meiotic phases that distinguish bivalents from the chromosomes of non-meiotic mitoses. They rapidly condense after the leptotene stage and a diplotene split makes its appearance; there follows a regular confused period; and the latter is in turn succeeded by an entirely typical series of diakinetic maneuvers. It would seem therefore that these characteristic prophase manifestations of meiosis are not at all conditioned by the phenomenon of pairing and, given certain basic conditions, the chromosomes run through the gamut of meiotic changes even when this most obvious feature of the meiotic prophase has been omitted.

### *Clumping and displacement of autosomes*

The clumping or aggregation of autosomes which occurs suddenly just before the first metaphase and is not completely abandoned until the spermatid stage, is one of the most striking features of the harlequin lobe. It is only natural to seek some interrelation between this anomaly and the failure of synapsis that precedes it, but the connection is certainly not a direct one. Thus the omission of pairing in Loxa (Schrader, 1945b) is followed by no clumping in the first division (although there are indications of "stickiness" in the second). The most decisive evidence against such an interrelation is furnished by Brachystethus (Schrader, 1946). There the clumping of autosomes is even more persistent than in the Discocephalini but an omission of pairing obviously cannot be held responsible since the autosomes undergo normal synapsis and form typical tetrads.

The occurrence of autosomal heteropycnosis in the prophase of *Mecistorhinus melanoleucus* can also not be held responsible for the later aggregation of autosomes. In *Mecistorhinus tripterus* the clumping is just as marked as in the first named species, but there is no heteropycnosis in the autosomes at all. Indeed, despite the peculiar maneuvers that such heteropycnosis brings about especially during diakinesis, the meiotic divisions themselves are not perceptibly affected. They are essentially the same, whether autosomal heteropycnosis be present or not.

In all these considerations of the clumping phenomenon it must be remembered that the mitotic apparatus remains normal and cannot be held directly responsible for the anomalies. The justification for this conclusion lies not only in the fact that chromosomal spindle fibers are regularly formed between the centrioles and the

clumped autosomes, but also in the perfectly normal spindle relations of the two sex chromosomes.

It is the orthodox behavior of the sex chromosomes that may furnish a clue to the autosomal behavior. As usual, both the X and the Y chromosome are persistently heteropycnotic and at first metaphase are fully condensed. The autosomes however depart from their usual cycle of condensation and diffuseness. Already at prometaphase their precocious reaction to the polar forces would suggest a condition at variance with that which normally obtains—perhaps a more advanced state of condensation. Such abnormal timing is indicated more directly when the disintegration of the nuclear membrane finally occurs. The autosomes have then begun to reverse the normal sequence of development and when they undergo the clumping reaction they are in process of returning to the diffuse condition rather than attaining their final condensation. It is this untimely regression in nucleination (for there appears to be only a slight uncoiling) that probably underlies their clumping.

Such a hypothesis implies a close correlation between the state of condensation and the mutual reactions of chromosomes. It may be objected that during a normal prophase, when the chromosomes pass through all stages of condensation, no such clumping ever occurs. But it must be remembered that during the entire prophase a nuclear membrane is present and there can now be little doubt that this exerts the most far-reaching influence on chromosomal behavior (Schrader, 1941). Indeed when the membrane finally breaks down there is in normal cells also an immediate clumping of chromosomes which do not separate again until they reach final condensation shortly thereafter. And in the normal telophase, when the chromosomes have started to return to the diffuse condition, there is again a tendency to clump. It is only after the new nuclear membrane has been formed that the chromosomes separate and scatter once more. The so-called mutual repulsion of the chromosomes in these Pentatomidae and perhaps other forms as well thus appears to occur only when they are fully condensed or else are contained within a nuclear membrane.

It is possible that here also lies the key to the extrusion of the autosomal aggregate from the midregion of the spindle. As has already been said, the orthodox behavior of the sex chromosomes in the same spindle proves that it is not in the spindle apparatus that an explanation is to be found. It is the autosomes and kinetochores that must be held responsible and the abnormality in their condition which results in clumping is probably also involved in their lateral displacement. That this aberrant condition is only temporary is indicated by the fact that there is a partial return to normal behavior in the second division. The aggregate of autosomes is then stretched between the two poles and, though still "sticky," many of its components become dissociated from each under the strain and again appear as individual chromosomes. By the time the spermatid stage has been reached their behavior appears to be normal and they scatter over the periphery of the nuclear membrane quite like the chromosomes of the neighboring lobes.

It is safe to assume that the establishment of the metaphase involves a set of precisely adjusted interactions between centers, chromosomes and kinetochores. That these interactions involve nothing more than a system of repulsions would seem to be very unlikely, and indeed the retention of the chromosomes within the compass of the equatorial plate almost demands forces of a positive nature. Whether these be contractile chromosomal spindle fibers or a zone of actual attraction in the equator or both, it is quite possible that an upset in the condition of one of the elements

involved might allow the forces of repulsion to gain the ascendancy.[2] On such a basis the regressive nucleination that is perhaps accompanied by a partial weakening of the kinetochores may well account for the expulsion of the autosomes in these Discocephalini.

*The preferential segregation of one autosome*

The special behavior of one of the autosomes and its association with the X chromosome has already been described. In analyzing its movements the following factors should be considered:

In the first division this chromosome shows no connection with either of the two sex chromosomes and its mitotic reactions are directed solely to the nearest pole. Its only distinction from the rest of the autosomes lies in a slightly greater condensation which is not very pronounced but may explain why it behaves more normally than they in this division. In the division of the smaller second spermatocyte this independence of the autosome is lost and to all intents and purposes it then becomes a satellite of the X chromosome.

The available evidence suggests that this chromosome represents a single chromatid of one of the large, univalent autosomes. It is probable that the two chromatids of this large univalent separate in a normal mitotic movement and that position in the aggregate determines which one frees itself and joins the two sex chromosomes at one pole. Its mate always moves to the opposite pole and with it goes the rest of the aggregate. The complications that attend these maneuvers arise primarily from the stickiness that tends to hold all autosomes together.

This provisional explanation of the peculiarities of the first division does not however throw much light on the division of the smaller second spermatocyte. The preferential segregation of the autosome is obviously a consequence of some interrelation with the X chromosome. To be sure, the two chromosomes are often connected by a Feulgen-positive strand or direct adhesion to each other. But before anaphase such a connection is sometimes also established between the autosome and the Y, and in still other cases there is no visible connection with either sex chromosome. Nevertheless, in the great majority of cases (about 95 per cent in *Mecistorhinus melanoleucus*) it is the X with which the autosome is finally associated at telophase and not the Y. The evidence as it stands therefore admits of only one interpretation which has only a few parallels in the literature—the preferential segregation of the autosome is due to some kind of attraction between it and the X (or, much more unlikely, its repulsion by the Y). A directed movement is involved in any case and since the X behaves just as it would in a perfectly normal cell it is the autosome itself which is primarily accountable.

*Evolutionary aspects*

It is a matter of surprise that so striking a feature as is comprised by the harlequin lobe should have escaped the notice of cytologists for so many years. To be sure it has so far been encountered only in the hemipteran family of Pentatomidae, but that particular group of insects has been subjected to more intense cytological study than almost any other comparable group except the orthopteran family of

[2] In a short article that has just come to hand, Östergren (Bot. Notiser, 1945) suggests that if the spindle is a tactoid such an interplay of forces may well be involved.

Acrididae. Work on some 70 species has been published and among the investigators have been such outstanding cytologists as Montgomery, Wilson, and Geitler. To now discover harlequin lobes in some eight genera and eleven species, widely scattered through the family, can hardly mean that their occurrence has simply been overlooked hitherto. We must seek an explanation elsewhere and in such a quest one cannot fail to be struck by one aspect of the situation. It so happens that despite the great number of species investigated all cytological work has been confined to Pentatomidae from the Palaearctic and Nearctic regions. The rich pentatomid fauna of the Oriental, Australasian, and Ethiopian regions has never been touched by cytologists, and only recently have Neotropical species been investigated. It is among the latter that I have encountered all the species that carry harlequin lobes and the question might well be asked whether the occurrence is not correlated with a tropical habitat. But that is not to say that all tropical Pentatomidae are characterized by such lobes for my own investigations have shown that a number of species of such genera as Alcaeorrhynchus, Arocera, Edessa, etc., do not possess it. Nevertheless the correlation is sufficiently striking to make an inquiry into the effects of various factors in tropical conditions on the development of reproductive organs well worth while.

The occurrence of harlequin lobes in four different species of the tribe Discocephalini collected in localities as far apart as Costa Rica and southern Brazil (about 5400 km. or 3350 miles) would seem to justify the conclusion that such a feature was present in the ancestral species of the tribe. Its absence in a fifth, a species of Platycarenus, might be attributable to loss in the course of evolution. But harlequin lobes are found also in some members of entirely different tribes such as the Halyini (unpublished) and the Pentatomini, and in such a remote form as Brachystethus (Schrader, 1946) its cytological character is obviously very similar to that of the Discocephalini. On the basis of our original reasoning we would thus arrive at the conclusion that a harlequin lobe characterized not only the first discocephalinid but was present already in the more remote ancestor of the whole family of Pentatomidae. Its sporadic occurrence at the present time may therefore be representative of evolutionary survivals that are possible only under certain conditions, though of course it is possible that the genetic basis for the harlequin lobe has existed for a long time but that this can express itself only under certain rather limited conditions. In either case, the deciding factor may well lie in the environment which happens to be more often favorable in tropical than in temperate regions.

Since the sperms of the harlequin lobe are ordinarily nonfunctional in the hereditary sense, it is obvious that in such species there is a very great loss of gametes. Natural selection might confidently be expected to eliminate so wasteful a development unless it also confers certain advantages that compensate for the loss of functional sperms. It has been suggested (Schrader, 1946) that these advantages may lie in the nucleoproteins that are carried into the eggs by supernumerary sperms. Polyspermy is of extremely common occurrence in the fertilization of insect eggs and it is probable that the nucleoproteins introduced by the extra sperms are utilized by the growing embryo. The sperms from the harlequin lobe are usually much larger than normal sperms and hence would contribute proportionately larger amounts of nuceloproteins. Species with harlequin lobes may therefore hold an advantage over those that lack them—an advantage that takes the form of an increase in materials that are of special value for the developing embryo. Certain it is that so

prosperous a species as *Mecistorhinus tripterus* which has a distribution from Vera Cruz, Mexico, to São Paulo, Brazil, has found the possession of a harlequin lobe no disadvantage.

The sporadic and yet widespread occurrence of harlequin lobes among the species of Pentatomidae argues strongly that we are dealing with a basic condition. The problem of why a certain lobe in the testis always takes a special course in its development may in the end be no different from that involved in such a case as the development of diverse types of digestive glands from the embryonic gut. In other words, we are confronted here, as well as there, by the old question of differentiation.

## Summary

1. In the testes of four species of the pentatomid tribe Discocephalini, the fifth lobe always shows a special type of meiosis.

2. There is no pairing in this lobe.

3. Just prior to metaphase I all the autosomes aggregate in one clump. The sex chromosomes behave quite normally through both meiotic divisions.

4. In the first division the X and Y divide equationally as in normal cells. One large autosome becomes dissociated from the aggregate and reaches the pole opposite to that attained by the rest of the aggregate.

5. A large and a small second spermatocyte result from this first division. In the small one, the single autosome goes to the same pole as the X in the great majority of second divisions.

6. In the division of the larger second spermatocyte, the aggregate is irregularly pulled into two groups.

7. Four main types of spermatids result: X + variable number of autosomes; Y + variable number of autosomes; X + one large autosome; Y. The last two types probably never reach the mature sperm stage.

8. It is pointed out that so firmly established a feature as this seemingly wasteful development in the fith or "harlequin" lobe must have some compensatory advantage, and its cytological and evolutionary significance are discussed.

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All figures are photographs from harlequin lobe; Figs. 72 to 77 inclusive from *Mecistorhinus melanoleucus;* Fig. 78 from *Mecistorhinus tripterus.*

Figure 72. Formation of equatorial ring (Hematoxylin).

Figure 73. Early metaphase I with the two sex chromosomes close together in the middle and the autosomal aggregate displaced to one side (Hematoxylin). Shown in two of the cells.

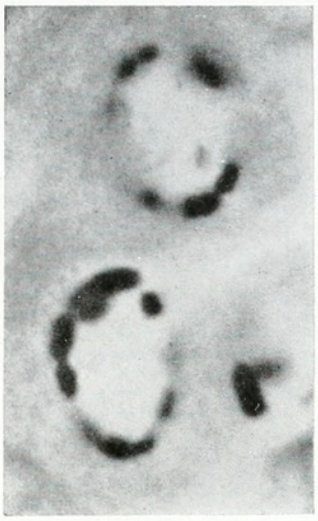
Figure 74. Beginning of anaphase I; X and Y in middle, large autosome beginning to protrude from displaced aggregate (Gentian violet).

Figure 75. Late anaphase I, photographed at two different levels; large autosome leaving aggregate, and an X and Y approaching each pole (Gentian violet).

Figure 76. Anaphase II (large cell); aggregate displaced to one side and X and Y separating to opposite poles (X to lower pole) (Hematoxylin).

Figure 77. Anaphase II (small cell); X with autosome going to upper pole and Y to lower pole (Hematoxylin).

Figure 78. Telophase II (large cell); aggregate becoming dissociated between separating X (lower pole) and Y (upper pole) (Feulgen).



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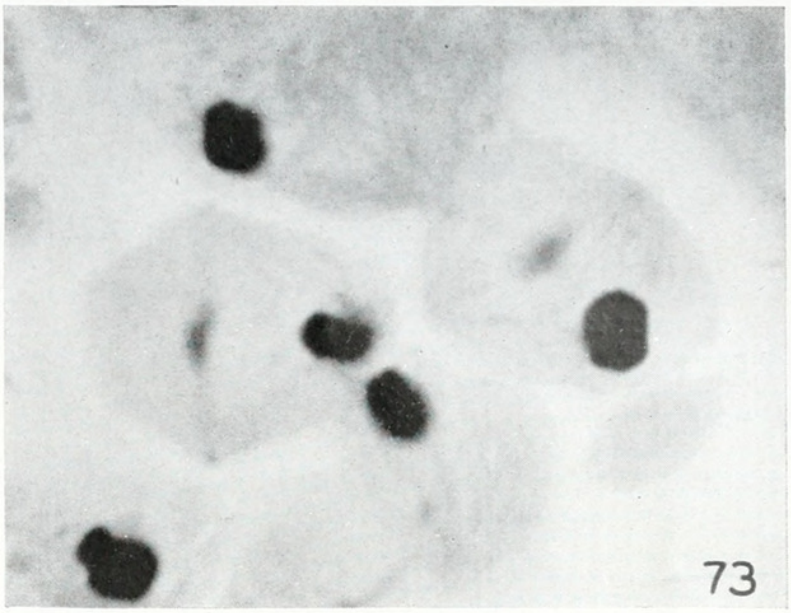

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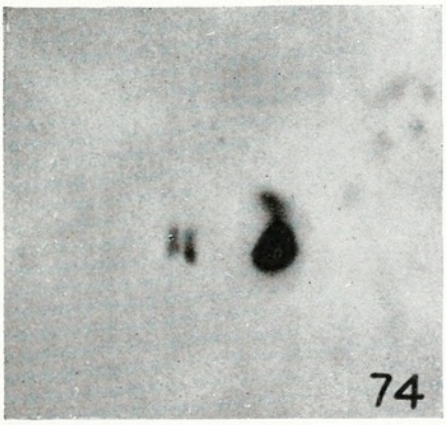
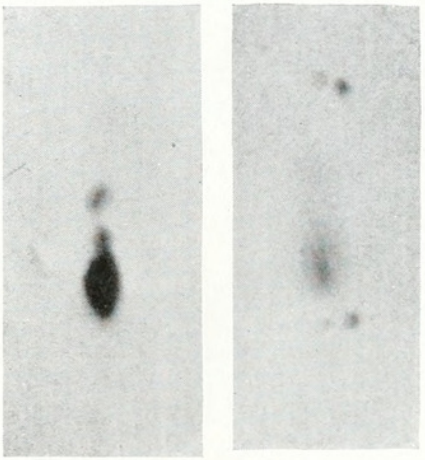
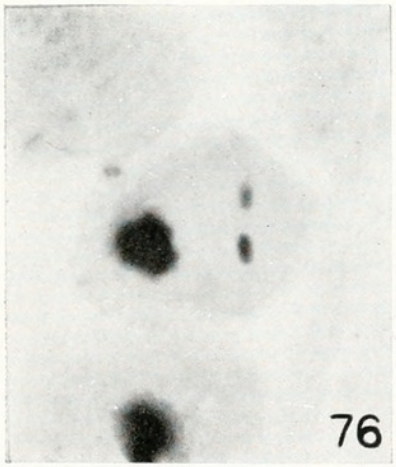


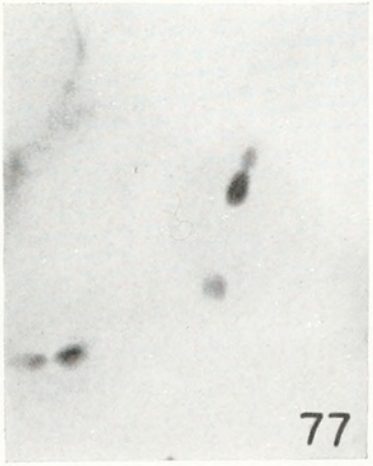
74



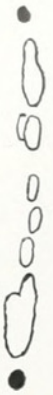
75



76



77



78

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