CHROMOSOMAL VARIATIONS IN THE EARWIG, ANISOLABIS ANNULEIPES LUCAS.

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The specimens for the following study were collected in the locality of New Orleans, La. and Mississippi College, Miss. The study, however, was based largely on the Louisiana material. The specimens were identified as *Anisolabis annuleipes* Lucas by A. N. Caudell, Bureau of Entomology, Washington D. C. The gonads were fixed in Flemming and the sections were stained with Haidenhain's hematoxylin and a few were counter-stained with eosin. All the work was done under the direction of Dr. Fernandus Payne to whom the writer is greatly indebted for helpful criticism and advice.

Several workers beginning with Carnoy in 1885 have described the spermatogenesis of the European earwig and there has been much disagreement in the descriptions and explanations of the various authors. Payne[1] reworking the spermatogenesis proves beyond a doubt that many irregularities do exist in this species and in this way accounts for some

Fig. 1-4. Spermatogonial metaphase showing 25 chromosomes.

of the disagreement of the preceding workers. Although the following study does not have a direct bearing on these explanations, nevertheless it does definitely show certain irregularities and probably with a more extended study of several species of earwigs we may find that most of the chromosomal irregularities of this whole group of insects will become understandable. The writer is at present working on a comparative study of the spermatogenesis of the earwigs.

Description. The spermatogonial number in *Anisolabis annuleipes* Lucas is 25 (figs. 1, 2, 3, 4). This was shown to be the case by a great many counts of clear metaphase plates.

During the growth period one or more dark staining bodies are seen in the nucleus (figs. 5, 6, 7). By following these bodies during this period and the early prophases it was found that one body very definitely continues as a bivalent chromosome. As shown in figures 8 and 9 the parts of the bivalent are not of equal size. The dark staining body and its changes in the growth period and early prophase is similar to that described by Payne in the European earwig except that he did not find the body bilobed. In the latter part of the prophase this unequal pair of chromosomes, which the body very definitely

[1] Payne, Fernandus. Chromosomal Variations and the Formation of the First Spermatocyte Chromosomes in the European Earwig, Forficula sp. Journal of Morphology, Vol. 25, No. 4.

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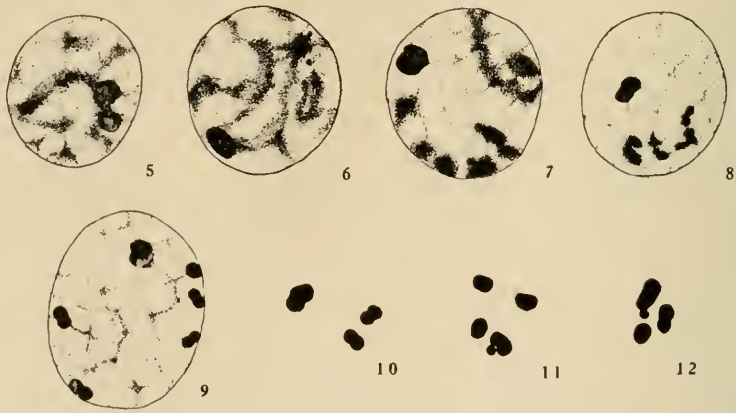
becomes, is seen occasionally to break up into a large bivalent and a small univalent chromosome (figs. 11, 12). The separation of the small univalent from large bivalent seems to take place in only a small percentage of the dividing cells while a condition similar to figure 10 is very often found.

With the spermatogonial count of 25 it would be expected that the metaphase counts of the first spermatocyte division would show 13, but such was not the case except in a small percentage of the counts. By counting only clear metaphase plates it was found that 92 per cent showed 12 chromosomes (figs. 13, 14). The study of the side views of the metaphase division showed clearly that the univalent chromosome did not lie in a different plane from that of the bivalent chromosomes nor did it pass to the pole before the division of the bivalents as has been described in several instances. In a very few of the cells examined it was found that a small univalent chromosome could be seen in the



Figs. 5-12. (5, 6 and 7), Growth period showing dark staining bodies; (8 and 9), prophase, dark staining body becoming unequal pair; (10), later prophase showing unequal pair; (11 and 12), small univalent separating from bivalent chromosome.

side views (figs. 17, 18). This accounts for the small percentage of 13 counts (figs. 15, 16).

The metaphase counts of the second spermatocyte division showed no such irregularity in chromosome behavior as that found in the first spermatocyte division. Of the 513 counts made of this division 280 showed 12 (figs. 32, 33) and 233 showed 13 (figs. 34, 35). A study of the side views of the division showed that all the chromosomes divided normally.

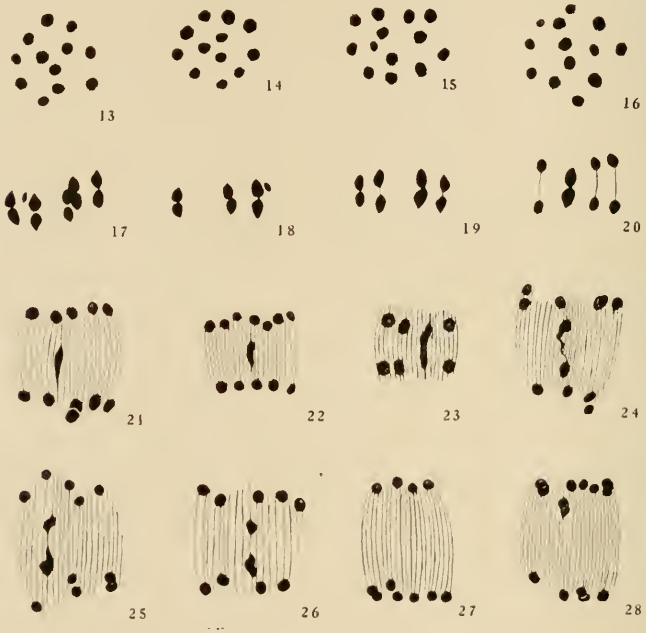
From the above facts it appears that the real problem here would be to account for the disappearance of the univalent chromosome that was present in the spermatogonial metaphase and its reappearance in the second spermatocyte division. The second spermatocyte counts showed nearly equal numbers of twelve and thirteen chromosomes. This is just what would be expected when the spermatogonial number is 25.

In the growth period and early prophase one of the dark staining bodies was seen to give rise to an unequal pair of chromosomes. This

unequal pair passed normally into the metaphase stage. With the condition described by Payne in mind, where the larger end of the unequal pair showed a bilobed appearance, it was thought that some such condition might be found here. A careful study of the side views showed no such condition, however. It was found that a slightly unequal pair did exist and that this pair started to divide shortly after the other pairs. Figures 11 and 12 of the prophase show the unequal pair breaking up into a large bivalent and a small univalent chromosome. Figures 17 and 18 show the small univalent chromosome in the metaphase. The separation of the univalent chromosome as shown in the above figures accounts for the normal thirteen count that was found in a small percentage of cases (figs. 15, 16). Another abnormality that was found in the side views of the first spermatocyte division was the large number of lagging chromosomes. This species differs in this respect from the European species in that the lagging chromosomes were found in the first spermatocyte division only, while in the latter they were found to occur in both spermatocyte divisions. One lagging chromosome was found in the second division but this evidence has been disregarded because of the probable pathological condition of the specimen. This pathological condition was quite evident in other cysts of the same testis. The form of the lagging chromosomes was seen to vary a great deal from a single elongated mass to that of a more or less trilobed condition (figs. 21-28). It was noticed as stated above that the unequal pair divided somewhat later than the other pairs. By tracing this unequal pair from the metaphase to the anaphase the evidence seemed to warrant the conclusion that the lagging chromosomes were these unequal pairs that were dividing irregularly. Figure 25 shows an unequal pair dividing into an upper single mass and a lower mass that is somewhat bilobed. Figures 23, 24 and 26 also indicate this condition. Figures 27 and 28 are serial sections of the same cell in anaphase. By counting the chromosomes in both figures the lower part shows definitely 12 chromosomes while in the upper part are 11 chromosomes and the bilobed chromosome that is slightly lagging. If this lagging individual is the unpaired univalent plus one half of the divided bivalent we have, then, 12 chromosomes passing to the lower pole and 13 to the upper. This is just the distribution that would be expected with the 25 chromosomes in the spermatogonial division. That the univalent chromosome becomes attached to one of the autosome pairs causing this lagging in the first spermatocyte division is further evidenced by the condition shown in figures 23 and 26. In figure 23 an enlargement on one side of the lagging chromosome is very noticeable. Figure 26 is a later anaphase than figure 23. Here the lagging chromosome has divided but the parts still lag behind the autosomes. The upper chromosome is much smaller than the lower and the irregular outline of the lower mass resembles the upper part of the lagging chromosome in figure 23. One suggestion the writer might offer to account for these lagging chromosomes is that it is determined by the position of the attachment of the small univalent chromosome to the larger autosomes. When the small chromosome becomes attached so as to adhere to both autosomes the three chromosomes assume the linear arrangement in early

anaphase and later the whole mass is drawn out into the characteristic strand shown in figure 21. Later anaphase show that these lagging chromosome groups finally divide unequally and pass to the poles losing their identity in the condensed mass of chromosomes of the late anaphase (fig. 29).

The above explanation of the lagging chromosomes is not offered to explain a condition of similar appearance found in other forms but applies only to *Anisolabis annuleipes.* The writer does not offer the suggestion to explain the presence of two lagging chromosomes which

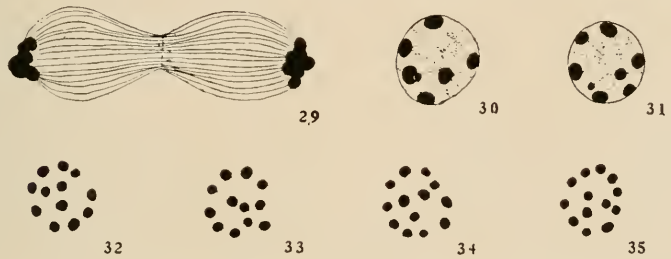


Figs. 13-28. (13 and 14), Metaphase polar view showing 12 chromosomes; (15 and 16), same showing 13 chromosomes; (17 and 18), side views showing small univalent; (19 and 20), side views of unequal pair; (21, 22, 23 and 24), side views of anaphase with lagging chromosomes; (25 and 26), division of lagging chromosomes; (27 and 28), serial section of a cell in later anaphase showing bilobed chromosome.

are only very rarely found in this species. Since the few cells with two lagging chromosomes seemed to degenerate in late anaphase the writer concludes that these cells fail to complete the maturation divisions.

Following the first spermatocyte division the nucleus passes through a short rest period before the second spermatocyte division (figs. 30, 31). A careful study of the nucleus from anaphase of the first to the metaphase of the second spermatocyte divisions was made. During the condensed appearance in the late anaphase the chromosomes could not be traced (fig. 29). In the reorganization of the nucleus the chromosomes separate and remain as distinct bodies distributed through the nucleus until the prophase of the second spermatocyte division. After this

reorganization a chromosome much smaller than the others can be seen. Figure 31 shows this small chromosome. This stage seems to be later than that shown in figure 30 where the small individual lies against the large chromosome. Conditions similar to these figures are very common in the cysts showing the rest stage. No great difference was found in the sizes of the chromosomes in the metaphase plates showing 12 chromosomes (figs. 13, 14) but in figures 15 and 16 showing metaphase plates with 13 chromosomes one small chromosome can be seen. This would indicate that the attached univalent was much smaller than the other chromosomes and this difference in size should make it noticeable at once when it became detached. On the basis of size the small body appearing in the rest period (figs. 30, 31) seems to be the univalent chromosome. Further study shows this small element behaving in the prophase of the second spermatocyte division as the other chromosomes. This seems to warrant the conclusion that the small univalent that disappeared prior to the first spermatocyte division became attached



Figs. 29-35. (29), Late anaphase showing chromosomes in condensed mass; (30 and 31), rest stage between first and second spermatocyte divisions showing very small chromosome; (32 and 33), second spermatocyte metaphase with 12 chromosomes; (34 and 35), same showing 13 chromosomes.

to one of the autosome pairs and remained attached until the reorganization of the nucleus in the telophase of the first spermatocyte.

Since the specimens were collected fairly late in the season some of the testes were filled with mature spermatozoa and many of the others had few cysts with cells in division. Although all the stages described above could be traced in many specimens there were a few that had several cysts in metaphase that permitted a large number of counts per individual. A description of one typical specimen with several cysts in metaphase might be of interest here. Specimen 40 seems to be of interest in this connection. Only one clear count of the spermatogonial metaphase could be made. It showed very definitely 25 chromosomes. The description of the growth period given above could be applied to this specimen (figs. 5, 6 and 8) although very few prophase groups similar to figures 11 and 12 were found. Here all of the 14 clear metaphase counts in the primary spermatocyte showed 12 chromosomes. The one cyst in the rest stage between the first and second spermatocyte divisions showed clearly many stages the close association of the small chromosome and the large one similar to figures 30

and 31. In the secondary spermatocyte there were 69 polar views of the clear metaphase plates. Of this number 37 showed 13 and 32 showed 12 chromosomes (figs. 32, 34 and 35). This is just what would be expected where there were very few cases of the prophase separation of the small univalent and the large bivalent chromosomes (figs. 11 and 12). The metaphase counts alone show very definitely that the univalent chromosome behaves irregularly prior to the second spermatocyte. No lagging chromosomes were seen in this specimen.

The above evidence leads to the conclusion that the small individual seen in the resting stage and early prophase of the second spermatocyte becomes attached to one side of the large chromosome pair sometime between the metaphase of the spermatogonial division and the prophase of the primary spermatocyte. Although this attachment has not been observed it probably takes place very early in the growth period. These attached chromosomes form the larger end of the unequal pair. Occasionally the chromosomes become detached allowing all pairs to divide normally while the univalent chromosome passes undivided to one pole giving the 13 chromosomes in the first spermatocyte metaphase. Nothing can be said with certainty as to the mode of attachment although the small chromosome seems to be plastered to the side of the larger chromosome so as to show no break in the general outline of the mass except for a slight enlargement (figs. 19 and 20). It might be expected that the attachment of the univalent chromosome to one-half of the autosome pair would make a very noticeable enlargement but such was not the case. Figures 17 and 18 show the univalent chromosome unattached but near one of the large autosome pairs. Assuming that the small chromosome was plastered to the upper half of the large bivalent chromosome the resulting mass would not be greater than the larger end of the unequal pairs shown in figures 19 and 20. After the condensed condition in the anaphase of the first spermatocyte division (fig. 29.) the chromosomes separate as individuals from this chromosomal mass and remain separated to divide normally in the secondary spermatocyte. This accounts for the unusual number of 12 chromosomes in the first spermatocyte and the normal number of 12 and 13 chromosomes that appears in the second spermatocyte.

It is interesting to note that the same numerical condition has been found in the distribution of the chromosomes of *Anisolabis maritima* by Kornhauser[2].

[2] Kornhauser, S. I. Cytology of Anisolabis maritima. Abstract of Papers, American Society of Zoologists 1920.