ON THE SPERMATOGONIAL DIVISIONS IN
AULARCHES MILIARIS, L.

BY T. RAMACHANDRA RAO, M.SC.,
Department of Zoology, University of Mysore, Bangalore.

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The remarkable constancy in chromosome number exhibited by Acrididae
has been well established by the studies of Prof. C. E. McClung and his
associates. Recently studies on the members of the sub-family Pyrgomor-
phinae have shown that there is an interesting deviation in the chromosome
number among them. All the forms collected from every part of the globe
possess 19 chromosomes in the males. The chromosomes are all rodshaped
and telomitic, except in the case of one of the specimens of the Mexican
Sphenarium, where a multiple chromosome occurs resulting in a complex
of 17 chromosomes (McClung, 1930). Owing to this smaller number and
the uniform telomitic nature, the Pyrgomorphine chromosomes are very
suitable for detailed studies on the chromosome structure and behaviour.
As McClung has suggested, here is "a form of chromosome relation of much
significance" which "should be studied as a type". Comparative studies
in the members of the group also seem to be very necessary. With
this aim in view Prof. J. J. Asana of Ahmedabad and the present author
have studied a number of Pyrgomorphinae. The following Indian genera
have come under the observation of the author: Aularches, Orthacris,
Colemania, Chrotogonus, Pecilocerus and Atractomorpha.¹

The present paper has within its scope only the spermatogonial divisions,
as found in Aularches. This genus is well suited for these studies on account
of the large size of the chromosomes. The aim of the author is to present
a description of the conditions found in this form so that they may
be later compared with those occurring in other grasshoppers. In broad
outlines they are very similar to the conditions prevailing elsewhere. But
Aularches provides strong evidence for the prevailing view of telophasic
duality, as also of the chromonema structure of the chromosomes. There-
fore in this paper special attention is paid to the following points: (1) to

¹ Brief observations on the chromosomes of Indian Pyrgomorphinae have been
read by Prof. Asana before the sessions of the Indian Science Congress, 1928 and 1930,
and by the present author on the Spermatogenesis in Orthacris, in January 1933.
General features have also been recorded in Current Science, 1, Nos. 2 and 4, 1932.
provide a broad outline of the mitotic cycle, (2) to test the validity of the chromonema theory, and (3) to determine the time when the longitudinal split becomes apparent. This genus also possesses some interesting conditions in the spermatocytes and spermatids which will be dealt with separately.

_Aularches miliaris_ is commonly found in the Western Ghats of India, feeding on coffee plants, and specimens were secured through the courtesy of the Entomology section of the Department of Agriculture in Mysore. The fixatives employed by the author were chiefly Flemming's weak solution and Carother's modification of Bouin's fluid. The latter was extremely successful and the majority of specimens was fixed in it. Hot water treatment before fixing in Benda's fluid as utilised by Sharp (1929) for plants was disastrous to the present material. A variety of staining methods was employed, chiefly, Iron-hæmatoxylin, Feulgen's method and gentian violet. The figures have all been drawn from preparations made from material fixed in Carother's fluid and stained in Iron-hæmatoxylin. Almost all the figures have been drawn from the same individual.

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**Observations.**

1. **Chromosome number, size, form, etc.—** _Aularches miliaris_ possesses 19 chromosomes in the males. They are all quite large and are rodshaped with telomitic spindle attachment. In size there is a gradual seriation so that it is practically difficult to identify each and every chromosome, but the largest and the smallest can be recognised with reasonable accuracy. During metaphases (Fig. 1) they are all seen quite easily, and the largest of them are slightly curved, possibly to be accommodated easily within the limited cell interior. The metaphase chromosomes exhibit no internal structure.

2. **Spermatogonial cells.—** The testis in _Aularches_ is made up of a large number of follicles, at the blind ends of which the spermatogonia can be made out. They are all closely packed together and can be differentiated into primary and secondary spermatogonia (Davis, 1908; O. E. Nelsen, 1930). The nuclei of the primary spermatogonia which surround the apical cell are large with a thin layer of protoplasm around them. The secondary spermatogonia arise immediately on the first division
of the primary ones, move away from the apical cell, become surrounded by a thin layer of connective tissue cells and proceed to divide. This method of formation of the spermatogonia is similar to those in the majority of grasshoppers and differs from the condition in the Tettigidae (Robertson, 1931) where the primary cells continue to be in relation with the apical cell, till the 32-cell stage, when they become separated from it. The spermatogonial cells undergo eight consecutive divisions leading to the formation of cysts of 256 cells. The protoplasm becomes smaller and smaller as the divisions follow in quick succession.

The earlier secondary spermatogonial cysts show that there is a regular arrangement of these cells in relation to each other. The cells are pear-shaped with their apices directed towards the centre of the cyst. The nuclei are situated in the broader region while in the protoplasm of the apical region of each cell, lies a faint more refractile body which, in some cases, extends into two adjoining cells. Evidently these are the "interzonal bodies" mentioned by Davis.

3. Anaphase.—The anaphase processes are practically the same as in all other organisms. Some structures observed by numerous workers on various plants and animals cannot be noticed here. The chromosomes take on a deep stain with very clear and sharp edges. The shorter chromosomes divide first and the longest chromosome last. In the late anaphase figure the longest chromosome is seen extending below the others (Fig. 2). As this has no mate, it is regarded as the sex chromosome. The ends of a large number of chromosomes were studied, to find if there is any evidence to show that the chromosomes are double at this stage. This search was unsuccessful though the appearance of the end of the long chromosome in Fig. 3 seems to suggest it. Occasionally some chromosomes were found to possess during later anaphase stages a wavy outline.

4. Telophase.—The first feature to be noticed after the chromosomes reach the poles, is that they are all regularly orientated, i.e., lie parallel to each other. This parallel orientation continues into the later phases, only to be lost in the final prophase stages when the nuclear membrane disappears.

In the early telophase stages the chromosomes gradually become less sharply outlined. Their uniform or rarely wavy contour of the previous stages give place to a clear twisted appearance, indicating that there is a regular spiral. In the longest chromosomes it may be noticed that there are four to five twists. The formation of the resting nucleus from the telophase chromosomes is chiefly the result of the diffusion of the chromosomes, each behaving independently. This independence is carried into the resting stage, where there are nineteen chromosome vesicles. The formation of
the resting nucleus is represented by the formation of a vesicle by each chromosome. This process may be understood by studying a number of stages in the telophase.

(a) In the first stage (Fig. 4), the chromosomes become slightly swollen and less faintly stained, and their outlines less sharp. They acquire a twisted appearance, but their internal structure is not yet resolvable. The difference in the lengths of the chromosomes continues to be visible. The nucleus is not yet organised. Polar views (Fig. 5) of this stage show the still cylindrical chromosomes closely packed together though with hazy outlines. Fig. 6 shows two chromosomes from a nucleus in the same stage which already show faint translucent spots inside them.

(b) In the second stage the chromosomes have more diffuse outlines. The spiral twisting of each is quite evident. For the first time they show a clear internal structure (Fig. 7). Certain regions are lighter than others and these vary in size. The outlines of the chromosomes are vague and the area of each is enlarged. The whole mass of chromosomes seems to be enclosed in the beginnings of a nuclear membrane. In polar views the chromatin is restricted to the centre of each chromosome and surrounded by a thin layer of a clear material. The chromatin itself shows an irregular outline. The lighter spaces observed in the side views are here seen to pass through the whole thickness of the stained chromatinic portion and to break through the chromosome boundary (Fig. 8). Figs. 9–13 show chromosomes drawn separately, showing these features more clearly.

(c) In the next stage (Figs. 14 and 15) the chromosomes appear of approximately uniform length, and their diffusion has gone a step further. Each chromosome has a thin chromatinic portion imbedded in a more fluid, less staining portion. The lighter regions are more pronounced and the stained portions thinner. The latter appear as two thin loosely intertwining threads. They are not altogether separate. Figs. 16–20 show the chromosomes of these stages. Owing to two facts, viz., the overlapping of the two threads in certain planes and the presence of cross connections, the intervening clear spaces might appear to be the results of alveolization. That they are not vacuoles but merely spaces between the spiral threads is very evident in Aularches. The spiral structure is best seen in Figs. 16–19, where at one end the doubleness is observed. In other words, the chromatin is "longitudinally" split. In some chromosomes the split is very marked while in others it is not sharp and precise.

(d) The next step in the formation of the resting nucleus is marked by the great diffusion of the matrix of the chromosomes, which in each case has a clear border abutting on that of its neighbours (Figs. 21 and 22). The
chromatin portions become very thin and the lateral projections more numerous. This thinning and branching of the two threads lead ultimately to the formation of a clump of fine threads in the centre of each chromosome. The doubleness of the chromatin becomes obscured on account of the great diffusion.

By this time the whole nucleus has assumed a roundish or oval outline, compared with the more lobate nature in the earliest stages. (Some resting nuclei, however, retain their lobate nature.) Davis (1908) says that in Dissosteria the vesicles are not quite independent of each other and that they are only so in the regions where the longer chromosomes project out. In Aularches it is obvious that the chromosome vesicles are quite independent of each other, throughout.

In this process of diffusion the sex chromosome behaves rather peculiarly. It is the first to diffuse out. In Figs. 14 and 15 the sex chromosome is seen to have already formed a vesicle of its own and the chromatinic portion has become very diffuse. This peculiarity of the sex chromosome can be observed in the prophase also where it is the last to become condensed and to assume the typical prophase form. Consequent upon this peculiarity the sex chromosome always offers a striking appearance in the late telophase and early prophase.

5. Interphase.—The interval between the telophase and the next prophase is very short in Aularches. The interphase (Fig. 23) is characterised by the presence of discrete and independent vesicles, in which the chromatinic portion becomes distributed. As no special technique was employed the interphase did not yield any internal structure to be studied. During the interphase, i.e., when the maximum diffusion takes place, the sex chromosome vesicle cannot be easily distinguished from the others.

6. Prophase.—The essential feature to be observed in the formation of the prophase chromosomes is, that they all arise independently within the limits of their own vesicles. The longest chromosome exhibits a slightly different behaviour from the others. It takes a slightly longer time to condense and forms a very characteristic figure in the early prophases. The

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There are apparently certain marked differences between the earlier and later spermatogonial cells in respect to the degree of diffusion during the interphases. The last spermatogonial telophase is, for instance, characterised by the almost total non-formation of vesicles. On the other hand, the matrices seem to fuse together. The chromosomes retain their characteristic orientation, i.e., with their ends resting on the opposite nuclear walls. The twisted nature of the chromosomes and the longitudinal split are as clear as in earlier cells. The sex-chromosome alone forms its own vesicle and persists during the interphase as a dark thin crescentic structure closely pressing the nuclear wall.
processes concerned in the formation of the prophase chromosomes seem to be somewhat the reverse of those in the telophase.

The orientation of the previous telophase is retained. On careful examination it is found that the chromatin of each chromosome is now really arranged in a very close spiral (Figs. 24, 25 and 26). The alveolar appearance is due to the presence of clear regions between the close spiral.

In a slightly later stage (Figs. 27 and 28) the spirally disposed threads gradually thicken and the coils become wider. In Figs. 29, 30 and 31 the two threads are widely separated in some regions and more approximated in others. Fig. 32 shows a polar view of the same stage. The chromosomes are seen to be independent of one another.

Fig. 36 shows a later prophase than that shown in Fig. 32. The chromatin threads are thicker and the space surrounding them clearer. Figs. 33, 34 and 35 which have been drawn from a slightly earlier stage show that the threads are clearly double. Fig. 34 represents a later stage of the condition shown in Fig. 29. As the prophase advances (Fig. 37) these spirals become wider and wider and the chromosomes themselves become thicker and longer.

Fig. 38 shows a nucleus in which the prophase chromosomes are almost completely formed although they are still in their own vesicles. The spaces inside the vesicles are quite clear suggesting that the chromosomes have formed at the expense of the karyoplasm.

The membranes of the vesicles disappear later and the prophase chromosomes lie free in the cytoplasm without any orientation or arrangement. Gradually they become thicker and the sister halves of each chromosome draw together and the split between them gradually becomes obscured.

The chromosomes of the late prophase and the metaphase do not show any internal structure at all, though it is warranted by inference to conclude that each sister half receives one of the thin spiral threads of the early prophase.

Discussion.

The points that require special attention in the foregoing account of the spermatogonial divisions in Aularches are:—1. The chromosomes exhibit a great individuality; during interphases they form independent vesicles. 2. The formation of the vesicles takes place by the limited diffusion of the chromosome matrices. 3. The more chromatic part of each chromosome is in the form of fine threads. 4. These threads—the chromonemata—appear in the telophases to be double and to be intertwined about each other; as telophase advances they become very thin, perhaps
anastomose and finally reach the limit of visibility. 5. They once again arise in the prophase as spirals entirely within the limits of their own vesicles and exhibit a double structure from the earliest stages.

The several studies on the chromosome vesicles in Orthoptera have shown their great importance as evidence for the continuity and individuality of chromosomes. This matter has been fully gone into by several authors, chiefly by McClung (1924, 1927), and need not be considered here again. But the observation that the vesicles are formed by the limited diffusion of the chromosome matrix offers some suggestions regarding the nature of the "Karyoplasm". Kaufmann (1926) and Sharp (1929) are inclined to the view that fluids enter the nucleus during the resting and the metabolic stages and that they are mingled with the chromosome matrix to form the transparent karyolymph. On the contrary, Martens (1922) believes the two substances to remain distinct. In *Aularches* during the resting stages the karyolymph of each vesicle is a homogeneous clear substance without any distinctions between the extra-nuclear fluids and the chromosome matrix. As the chromosomes must grow after each division, the extra-nuclear substances are expected to be converted into the substance of the chromosome matrix and absorbed into the chromosomes in the prophase. In figures of late prophases it is commonly seen that the vesicles are very transparent, while the spiral chromosomes within them are much condensed. The degree of transparency of the vesicle is directly proportional to the condensation and thickening of the chromosomes. These are suggestive of the idea that the old matrix along with the extra-nuclear substances are absorbed into the meshes of the chromonemata. In *Aularches* and other grasshoppers there is no question of the intermingling of the matrices of the various chromosomes as they remain distinct throughout. In the last spermatogonial division, however, a single nucleus is formed by all the euchromosomes and a combined karyolymph is therefore present. The sex-chromosome, however, retains its separate vesicle.

As to the occurrence of the telophasic split several opinions are held which are closely bound up with the question of the chromonema hypothesis. The weakest point in this hypothesis, according to Wilson (1925), was the telophase chromonema, but during the last few years increasing evidence has been adduced by many authors, on this point, chiefly by Kaufmann (1926), Sharp (1929), Telezynsky (1930), Hedayetullah (1931), Perry (1932) and Gates (1933). These plant cytologists have developed the idea that the chromonemata are double during all stages of mitotic cycle, and they thus support the observations of McClung (1927, 1932) and Robertson (1931) on the occurrence of the telophase split in Orthopteran chromosomes.
Belar (1929), Darlington (1932) and Belling (1933) have, however, expressed opinions against the occurrence of the telophasic split. Darlington's precocity theory of meiosis is partly built upon the idea of the singleness of the chromosomes before the resting stages and accordingly he regards all observations of splits in telophase as optical effects. Belling believes that there is no splitting of the threads at all, for he maintains that chromomeres are the only structures that divide and that fresh connecting fibres are formed *de novo* at every division to unite the newly formed sister chromomeres. This division of the chromomeres is supposed to take place only in the middle prophase. Huskins (1933) and his collaborators have brought forward evidence that Darlington's precocity theory is insufficient to explain all observed phenomena, and regard that the actual initiation of splitting occurs one division cycle earlier in the case of somatic nuclei, while in the premeiotic division alone the splitting is belated resulting in synopsis and other meiotic processes. Huskins accepts the data of Sharp, Hedayetullah and others regarding the splitting of chromonemata during "pro-metaphase" in preparation for the next division.

From the above short résumé of the current views on the subject it is seen that there is a great divergence of opinion among competent cytologists and it is a difficult matter to answer all objections. Regarding the alveolization hypothesis, Sharp has given strong reasons for abandoning his own previous views, and the matter has also been fully gone into by Hedayetullah and Gates. Belling's explanation of the structures seen in the telophase is that they are the effects of long fixation. That such structures are not seen in quickly fixed smears cannot be valid proof against their occurrence, which is only brought to light by a suitably long fixation. Recently Baumgartner (1933) has shown that fixed preparations in some insects are very similar to the conditions in the living nucleus. Even admitting that the observed structures are the effects of fixation, it remains to be satisfactorily explained why similar structures are brought into being by different fixatives. Without passing any opinion on the hypotheses of Darlington, Belling and Huskins all of which have their points of merit, it can be said, from observations of actual structures, that in grasshoppers the chromosomes exhibit a duality during the telophases in that there are two intertwining chromonemata. This confirms the observations of McClung and Robertson. The exact manner and time of the splitting of the chromonemata could not be decided from the present study.

The spiral structure of chromosomes is a well-established feature, though such a chromatin pattern "either is not uniform throughout the various species, or if so it has sometimes been misinterpreted" (McClung, 1927).
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Sutton (1900), Davis (1908), Wilson (1925), Belar (1929) and others have demonstrated that in many Orthoptera the fine contorted threads of the prophase arise by uncoiling and unravelling from massive larger bodies, often within the limits of their own vesicles. The present study indicates that from the earliest stages they exhibit a double structure. Figs. 29, 30 and 31 drawn from certain coils of early prophase show that there are two chromonemata which are closely approximated in certain regions and widely separated at others. Due to their becoming thicker and less coiled they are drawn more towards each other giving an impression in middle prophases that the chromosomes are not yet split. But Figs. 33, 34 and 35 clearly show that the apparently single coil is really composed of two moieties. In later prophases the double nature is very evident and needs no mention. In the earliest prophase stage, i.e., when the coils suddenly emerge from the vesicles, observation of detailed structure is impossible, but from a comparison with the immediately following stages it can be reasonably assumed that the threads are double in them also, and that the prophase split is a direct descendant of the one observed in the telophase.

The first account of spermatogenesis in a Pyrgomorphine was given by Machida (1917) who dealt with the spermatogonial divisions of *Atractomorpha* only briefly. He observed the telophasic split but made the error, usual at that time, in regarding that the longitudinal split between the synapsing homologues in the first spermatocyte to be a descendant of the telophasic split of the last spermatogonia. He also believed that in *Atractomorpha* the chromosome number was liable to certain variations. Recent studies on a larger number of Pyrgomorphinae have indicated that the number (19) is very constant, and the general cytological condition is similar to that in the other groups of Acrididae. Numerical variations, if any, (as in *Sphenarium*) proceed on the same lines as in the other sub-families.

**Summary.**

1. There are 19 telomitic rod-shaped chromosomes in *Aularches*.

2. The spermatogonial cells and the behaviour of the chromosomes in them are similar in general to the same processes in other grasshoppers.

3. There is strong evidence for the chromonema theory of the structure of chromosomes.

4. The chromonemata are double in the telophases and become very thin and reach the limit of visibility during the resting stage. This supports the observations of Robertson, McClung and others on the telophase splits in grasshoppers.
5. The chromosome vesicles are formed in the interphase due to the limited centrifugal movement of the chromosome matrices.

6. The chromonemata exhibit a spiral structure in the prophase.

7. The earliest prophase stages show the chromonemata to be double.

8. The chromonemata gradually thicken and uncoil leading to the late prophase chromosomes.

Addendum.

Three recent communications of considerable interest have appeared on the morphology of chromosomes, which can only be briefly mentioned here. Mather and Stone (J. Genetics, 28, No. 1, 1933) have sought to disprove the existence of telophasic split by the study of certain chromosomal aberrations. They find that in irradiated cells, a number of aberrations arise during the resting stage, and if the abnormalities occurred before the chromosomes were split, both the resulting chromosomes ought to show them. They have found this to be always true, there being no irregularities such as should result if the split occurred before the resting stage. Huskins (J. Genetics, 29, No. 1, 1934) has further developed his ideas regarding the unified theory of mitosis and meiosis and has shown that in somatic mitosis the chromosomes are effectively split one division cycle earlier so that the telophase chromosomes must be regarded as double. Koshy (J. R. M. S., 53, No. 4, 1933) has elaborated the chromonema theory by detailed studies on the somatic chromosomes of Allium and has confirmed the observations on the duality of chromonemata. He has further shown that the twists of the chromonemata in the two limbs of the chromosomes are in opposite directions, the null point corresponding with the attachment constriction, and that this reversal of twists is essential in the mechanism of mitosis. While Mather and Stone believe that the attachment constriction plays a fundamental part in the mechanics of cell-division, Koshy regards the anaphase movements to be entirely due to the pull exerted by the spindle fibres. These points are receiving further consideration to see how far they are applicable to Orthopteran chromosomes.

REFERENCES.


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EXPLANATION OF FIGURES.

The figures were all drawn with Zeiss apochromatic oil immersion objective, N.A. 1.3 and Zeiss compensating ocular x20 or x10 with the aid of Zeiss
camera lucida at table level. Figs. 1 to 4 are drawn at a magnification of \( \times 1575 \), Figs. 29, 30 and 31 at \( \times 3940 \) and the rest at \( \times 3150 \).

Fig. 1.—Spermatogonial metaphase plate.
Fig. 2.—Anaphase.
Fig. 3.—A few chromosomes of late anaphase.
Fig. 4.—Early telophase.
Fig. 5.—Polar view of early telophase. The sex-chromosome seems to be in an advanced state.
Fig. 6.—Two chromosomes of a slightly later stage than Fig. 5 showing hazy outlines and translucent spots.
Fig. 7.—Lateral view of telophase.
Fig. 8.—Polar view of a similar stage as Fig. 7.
Figs. 9, 10, 11, 12 and 13.—Some chromosomes drawn separately to show the spiral arrangement of chromatins.
Figs. 14 and 15.—Later telophase stages.
Figs. 16, 17, 18, 19 and 20.—Individual chromosomes drawn separately to show the spiral arrangement of the chromonemata.
Fig. 21.—Later telophase showing the formation of vesicles.
Fig. 22.—Very late telophase showing the double threads in the vesicles.
Fig. 23.—Interphase.
Fig. 24.—Very early prophase.
Figs. 25 and 26.—Early pro phases showing dense spirals.
Figs. 27 and 28.—The spirals have become clearer.
Figs. 29, 30 and 31.—Some chromosomes of a similar stage as Figs. 27 and 28, showing the double spirals.
Fig. 32.—Polar view of early prophase.
Figs. 33, 34 and 35.—Three chromosomes of a slightly later stage showing the doubleness in certain places.
Fig. 36.—Mid prophase. The sex-chromosome is still a dense coil.
Fig. 37.—Late prophase.
Fig. 38.—Late prophase. The vesicles have not yet broken down.