Unusual Type of the Nucleolus Observed in a Bug, *Acanthocoris sordidus*[1)]

By

Tosihide Yosida

Zoological Institute, Faculty of Science, Hokkaido Imperial University

(*With one Plate and 13 Textfigures*)

While it is a usual phenomenon that the nucleolus disappears by the time of formation of the equatorial plate in the course of the cell division, a number of unusual cases have been recorded in higher plants, which show that the nucleolus remains *in situ* without disintegration even in metaphase (Yamaha and Sinotô '25, Zirkle '28, Christoff and Gentscheff '32, Föyn '34, etc.). The majority of cases so far reported concern with the somatic mitosis, and there has been little known regarding the taking place of the related event in the meiosis. In the course of study, working with hemipteran chromosomes, the author met with a clear example of the persistent nucleolus occurring in the meiotic division of a bug, *Acanthocoris sordidus* Thunberg. Though the evidence is to offer only one example of the persistent nucleolus, publishing these data may not be entirely uncalled for by reason that the phenomenon under consideration deals with the meiosis, not the mitosis as formerly reported, and further that the reported cases of this kind are previously very rare in animals.

Prior to this study, the chromosomes of the present species have been investigated by Toshioka ('35), reporting the existence of some supernumerary chromosomes, but he failed to find out the unusual nucleoli as described in this paper.

Before going further, the author's hearty thanks are due to Dr. S. Makino under whose direction the work has been carried

---

1) Contribution No. 196 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

out. The work was supported by a grant from the "Nippon Gakuzyutu Sinkokwai" given to Dr. Makino, for which a grateful acknowledgement is made.

Method: The testis of *Acanthocoris sordidus*, a species of the Coreidae, fixed with Allen-Bouin's solution provided the material for the present study. The sections were prepared following the usual paraffin technique. For the general study of the chromosomes the staining of the sections was subjected to the iron-haematoxylin method after Heidenhain with the counter-stain of light-green. With the special purpose to give a sharp differentiation of the nucleolar element from the others, 1) Feulgen's basic fuchsin technique with light-green, 2) a single staining method of safranin, and 3) Newton's gentian violet method were also employed.

Observations

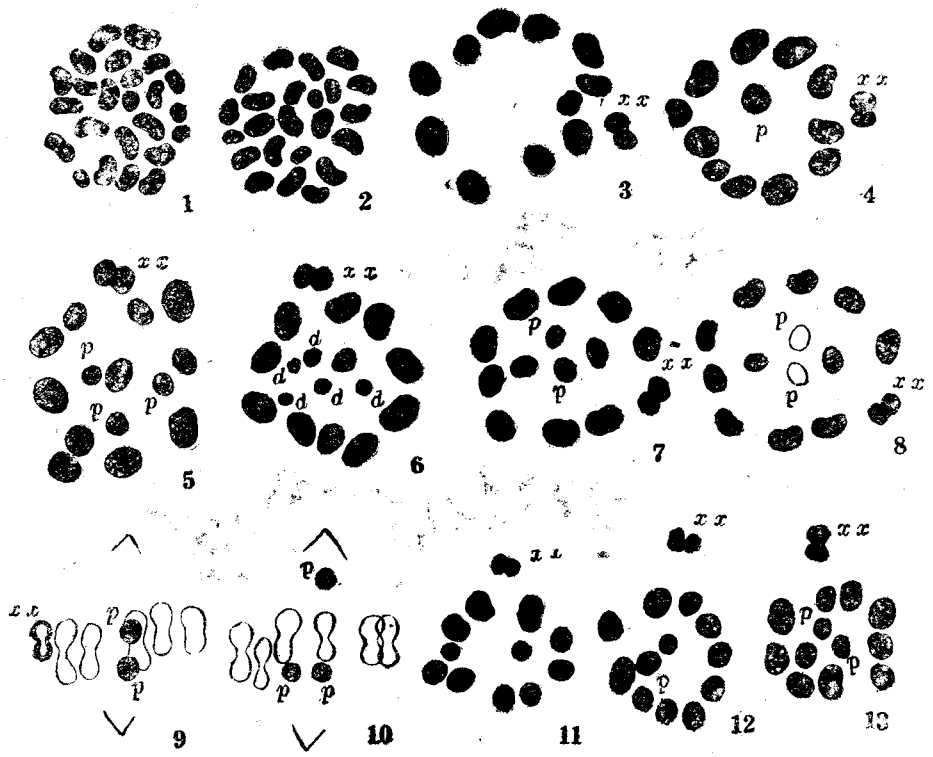
I. Spermatogonial division

The metaphase plate of the spermatogonial division invariably shows 24 chromosomes as $2n$ in every case under observation (Figs. 1–2). Specially noteworthy is the fact that there is no evidence for the presence of nucleolar element in the equatorial plate of the spermatogonial division. By application of any special staining method no kind of the nucleolar body could be demonstrated. Of twenty four chromosomes, two represent X-chromosomes, being certainly ascertained after the examination of meiotic phenomena. Thus, the present species was found to posses the XX-0 type of the sex-chromosome mechanism in the male, though the X-elements could not be pointed out with certainty on account of the absence of differential characteristics from the other autosomes.

II. Spermatocyte division

Careful examination of the first division reveals that, so far as primary spermatocytes are concerned, there are found in the testis two kinds of the equatorial plate as regards the distribution of the nucleolus. One of them contains no nucleolar body; hence the number of chromosomes counted in the equatorial plate of this kind

was found to be 12. As seen in Fig. 3, the autosomal bivalents lie in a circular manner in the equatorial plate, with the X's in close contact occupying the eccentric position. The other kind of the equatorial plate is remarkable in showing a certain number of nucleoli. The number of nucleoli observed is variable according to



All figures are camera-lucida drawings taken under magnification of 3500 times.

Figs. 1-2. The chromosome groups of spermatogonia. Fig. 3. The primary spermatocyte metaphase containing no nucleolar body. Figs. 4-6. The equatorial plates of the primary spermatocyte containing nucleoli, from one to five in number. Fig. 7. The equatorial plate of the primary spermatocyte stained with the iron-haematoxylin method. Two nucleoli are cleary recognizable. Fig. 8. The same plate re-stained with Feulgen's method with light-green. The nucleoli show an affinity to light-green. Figs. 9-10. Anaphasic figures of the primary spermatocyte showing at random migration of nucleoli. Fig. 11. The equatorial plate of the secondary spermatocyte having no nucleoli. Figs. 12-13. The metaphase plates of the secondary spermatocyte including one and two nucleoli respectively.

the equatorial plate, ranging from one to six. Fig. 4 is the plate in which a single nucleolus is present. In the plates shown in Fig. 5 and Fig. 6, there are three and four nucleoli respectively. According to the increase of the number, the nucleolus appeared becomes smaller in size. They assume in every case a round or oval outline and are always distributed in the inner area of the equatorial plate surrounded by the chromosomes.

In the iron-haematoxylin preparations the nucleoli generally stain as deep as the chromosomes, and therefore, to draw a sharp distinction between them is very difficult. Only due to the application of differential staining methods the bodies lying in the inner area of the metaphase plate are to be certainly demonstrated as nucleoli. By the use of safranin the nucleolar bodies are less stainable than the chromosomes which are deep red. Newton's gentian violet method is likely to give a similar reaction. Even in haematoxylin preparations a few cases were met with, where the nucleoli were more faintly stained than the chromosomes, giving a clear differentiation between them. The most sharp and full demarcation of the nucleolus from the chromosomes was obtained by application of Feulgen's basic fuchsin method with a counter-stain of light-green. With the adequate use of this method the nucleolus gives a typical acidophilic, while the chromosomes are basophilic. Fig. 7 is the equatorial plate obtained from a preparation stained with iron-haematoxylin and clearly shows 14 elements (see also the photomicrograph, Fig. 21). After being decolourized this preparation was re-stained by Feulgen's method with light-green. In the latter preparation the equatorial plate above concerned shows 12 elements which give basophilic reaction, while two bodies lying in the central position of the plate show an affinity to light-green (see Fig. 8 and its photomicrograph shown in Fig. 22). By this way it is proved beyond question that the two bodies of acidophilic nature are nothing other than nucleolus.

At anaphase of the first division, the migration of the nucleolus to the poles is entirely at random. They go undivided to one of the poles independently of each other (Figs. 9–10). Therefore, in some cases the nucleoli run together to one and the same pole, while in other cases they travel towards the opposite poles. As a consequence of the random distribution of the nucleoli in the first

division, various kinds of secondary spermatocytes, as regards the number of nucleoli contained, are to be produced. Fig. 11 is the equatorial plate of the secondary spermatocyte having no nucleoli and shows 12 chromosomes only. The metaphase plates shown in Fig. 12 and Fig. 13 include one and two nucleoli respectively. In the second division the nucleoli also go entire towards one of the poles at random, as done in the first division.

Remarks

Prior to this study, the chromosomes of *Acanthocoris sordidus* have been investigated by Toshioka ('35), with the result that a certain number of supernumerary chromosomes were observable in the primary spermatocyte. By the present investigation it becomes evident that the bodies noted as the supernumerary chromosomes by him are nothing other than the persistent nucleoli, as given in details in the foregoing description. In animal cells, the occurrence of persistent nucleoli has early been reported by Arnold ('09) in a Coleopteran insect, *Hydorophilus piceus*. He described a nucleolar body persistent up to the metaphase stage of the primary spermatocyte, but the reported evidence is very obscure. Recently Kawaguchi ('34) mentioned a clear example of the persistent nucleolus in the meiotic division of the hybrid between *Antheraea pernyi* (♀) and *A. yamamai* (♂), tracing to some extent its behaviour during spermatogenesis. In the latter case a single nucleolar body, irregular dumb-bell in shape, always appears in the cytoplasm of the primary spermatocyte. In the majority of cases recorded in plants, the persistent nucleolus is single in number and assumes in irregular massive form. The present species is quite remarkable in furnishing the fact the nucleoli always assume a characteristic, round or oval shape, and that they scatter in the inner area of the metaphase plate surrounded by the chromosomes. The author also found a like case in another species of Hemiptera, *Coptosoma punctissimum*.

With respect of the origin and fate of the persistent nucleoli in this species, the present study furnishes nothing to be stated here with certainty. It is quite curious that the existence of these nucleoli is confined to the meiotic divisions and there is no evidence for the presence of such elements in the spermatogonial division.

In animals, the relation between the formation of nucleoli and the chromosomes has not been fully researched out. The problems relating to the nucleolus covering its nature, behaviour and other cytological phenomena may be an important subject to concern future animal cytology.

Summary

Acanthocoris sordidus, a species of Hemiptera, was found to posses 24 chromosomes in diploid and 12 in haploid in the male germ-cell. The XX-0 type of sex-chromosome in the male was ascertained to occur.

The extra bodies which assume round or oval outline and vary in number from one to six, were found to exist in the metaphase equatorial plates of the primary and secondary spermatocytes. By the application of Feulgen's basic fuchsin technique with light-green and also by use of some other differential staining methods, it became certain that the bodies under question were true nucleoli.

The nucleoli are always found lying in the inner area of the metaphase equatorial plate being surrounded by chromosomes, and show no sign of disintegration throughout the meiotic stages, being not lost from sight. In division the migration of the nucleoli to the poles is entirely at random. They go undivided to one of the poles independently of each other.

It was found that there is no evidence for the presence of the nucleolus in the metaphase plate of the spermatogonial cell. In every case examined the spermatogonium showed 24 chromosomes at metaphase.

As regards the origin and fate of the persistent nucleoli here observed, nothing can be stated at present.

Literature

ARNOLD, J. J. 1909. The nucleolus and microchromosomes in the spermatogenesis of *Hydrophilus piceus* (Linné). Arch. Zellf. 2 : 181–190.

CHRISTOFF, M. and GENTSCHEFF, G. 1932. Untersuchungen über den Nukleolus während der Kernteilung. Zeit. Zellf. mikr. Anat. 15 : 540-546.

FÖYN, B. 1934. Lebenszyklus, Cytologie und Sexualität der Chleorophycee *Cladophora suhriana* Kutzing. Arch. Protstenk. 83 : 1-56.

KAWAGUCHI, E. 1934. Zytologische Untersuchungen am Seidenspinner und seinen Verwandten II. Spermatogenese bei *Antheraea yamamai* Gmerin, *Antheraea pernyi* Gmerin und ihren Bastarden. Jap. Jour. Genet. **10**: 135-151.

TOSHIOKA, S. 1935. Hemipteran insects as the material for study of idiochromosomes. (In Japanese). Bot. & Zool. 3: 1841-1846.

YAMAHA, G. and SINOTÔ, Y. 1925. On the behaviour of the nucleolus in the somatic mitosis of higher plants, with microchemical notes. Bot. Mag. (Tokyo) **39**: 205-226.

ZIRKLE, C. 1928. Nucleolus in root tip in *Zea mays*. Bot. Gazet. **86**: 402-418.

Explanation of Plate XXXI

Photomicrographs taken with the aid of "Leitz-Makam," under magnification of 2000 time. (Dr. S. Makino *photo*.)

Fig. 14. The equatorial plate of the primary spermatocyte containing a single nucleolus.

Fig. 15. The same having two nucleoli.

Fig. 16. The same having three nucleoli.

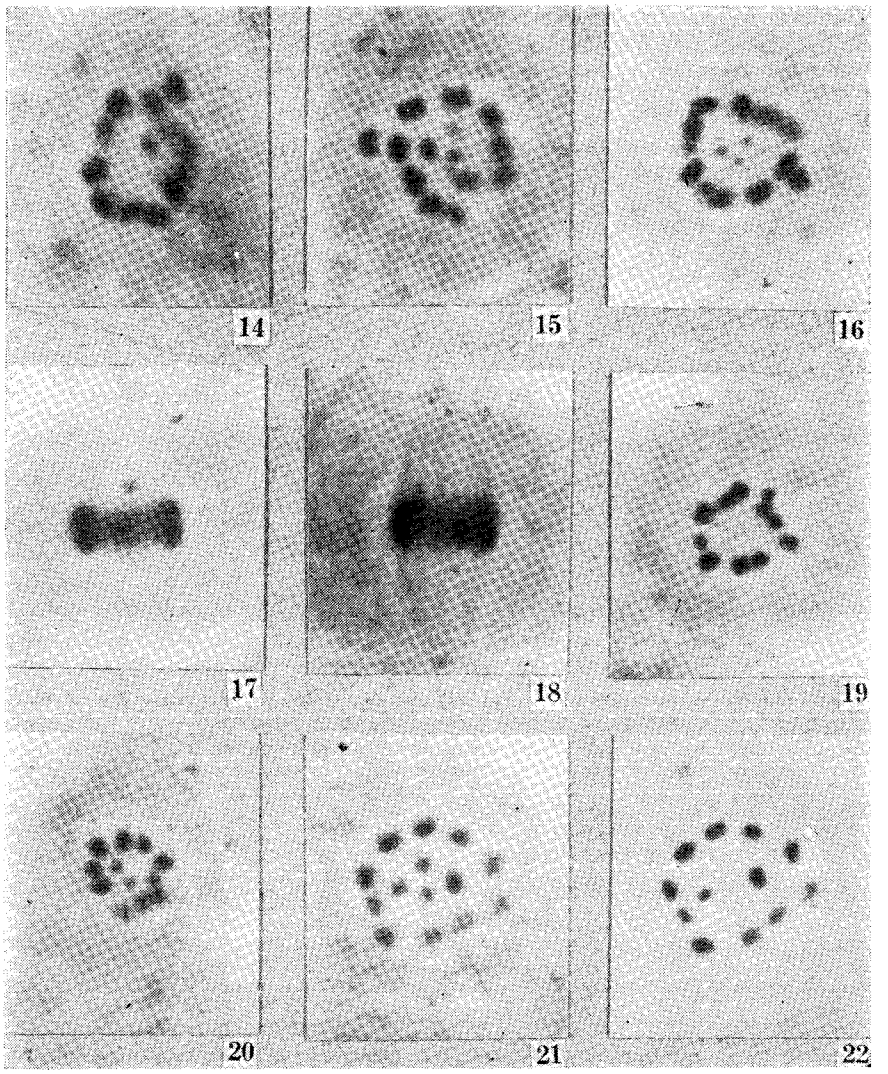
Figs. 17-18. Migration of the nucleoli at anaphase of the first division.

Fig. 19. The metaphase of the secondary spermatocyte showing no trace of nucleolus.

Fig. 20. The same containing a single nucleolus.

Fig. 21. The metaphase of the primary spermatocyte containing two nucleoli taken from the haematoxylin-preparation. The same as Fig. 7.

Fig. 22. The same plate re-stained with Feulgen's method. The nucleolar bodies did not stain at all in this case.



T. Yosida: Unusual nucleolus in Acanthocoris sordidus