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Author(s)	MAKINO, Sajiyo; KICHIJO, Hisao
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FEMALE HETEROGAMETY IN THE TRICHO-  
PTEROUS INSECT, *STENOPSYCHE*  
*GRISEIPENNIS MACLACHLAN*<sup>1)</sup>

BY

Sajiro MAKINO and Hisao KICHIJO

(With 3 Figures)

In recent years the close relationship between the orders of Lepidoptera and Trichoptera has been established by two cytologists, KLINGSTEDT and PCHAKADZE, from different standpoints of chromosome morphology. The former author (1928, 1931, 1932) studied the chromosomes of two species of Trichoptera, *Limnophilus decipiens* and *L. lunatus* conclusively, and discovered the sex chromosome which is unpaired in the female cell but coupled in the male with indisputable clearness, proving the striking similarity with Lepidoptera in respect to female heterogamety. The latter investigator (1930), on the other hand, investigated the chromosomes of twenty four species of Trichoptera covering eight families. He concluded that the Trichoptera form a group standing in the closest relation to the Lepidoptera in the morphological characters of chromosomes, *i. e.*, number, form and arrangement, referring to the conclusion of BELIAJEFF (1930) who studied the Lepidopteran chromosomes comparatively extending over thirty eight species.

So far as the sexual difference of chromosomes is concerned, however, the evidence found in *Limnophilus* by KLINGSTEDT (1928, 1931) is still a unique example throughout the order Trichoptera. In order to examine whether a similar fact may be found in other species included in that order, the present study has been undertaken during the last year.

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### Material and Methods

*Stenopsyche griseipennis* MACLACHLAN with which the present paper is concerned, is a common species belonging to the family Stenopsychidae and widely distributed over Asia. In Japan, it distributes, according to KUWAYAMA (1930), from Sakhalin in the north to Kyushu in the south as well as to Korea.

The larvae used for the present study were collected by the authors in a clear stream at a suburb of Sapporo at the middle of May, 1933. The testes and the ovaries taken from the larvae of various sizes comprise the material. For fixing, HERMANN'S and CARNOY'S fluids were employed. Sections were cut 7 micra thick and stained with HEIDENHAIN'S haematoxylin using light-green as the counterstain.

The figures were drawn with an ABBE drawing apparatus at the level of the desk on which the microscope was set, a 1.5 mm. ZEISS Apochr. obj. and a K 20×Oc. being used.

### Observations

#### *The chromosomes of the female cells*

For ascertainment of the chromosome constitution in the female, the somatic cells constituting the epithelium of the follicle were chosen as the material, on account of facility of obtaining good figures of chromosomes. Fortunately numbers of cells were in process of division, and available for the purpose of counting the complete number of chromosomes and of comparing the morphological charac-

ters, since the individual chromosomes are fairly arranged showing no indistinct overlapping. Examples are shown in Fig. 1, *a-f* (metaphase polar views). The chromosomes are all short rod-shaped, more or less curved, and show no marked size differences from one another. In form and arrangement they seem to be quite similar to those found in the Trichopteran species already studied (KLINGSTEDT, 1928, 1931; PCHAKADZE 1928, 1930; LUTMAN 1910).

In every equatorial plate examined, the number of chromosomes is twenty five; this must be the definitive number in the female cells. By picking out every two homologous chromosomes, according to size and form, it is found that the smallest one remains unpaired, as indicated by *z* in Fig. 1.

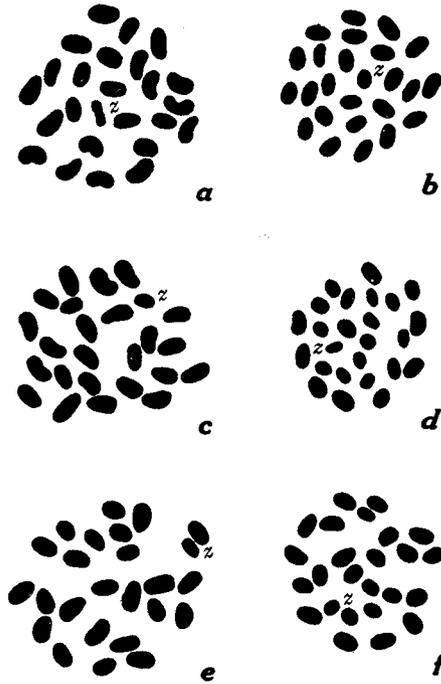


Fig. 1, *a-f*. Female diploid chromosome groups, from follicle cells, 25 chromosomes in each.  $\times 4200$ .

#### *The chromosomes of the male cells*

*The spermatogonium.*—In the prophase stages of the spermatogonia the nucleus contains one or occasionally two plasmosomes which are round-shaped and conspicuously large in size (*p* in Fig. 2, *a*).

In the metaphase the chromosomes take arrangement characteristic and similar to known cases of the Trichoptera. They never lie with their long axes radially against the center of the equatorial plate, showing the same appearance as found already in the female cells (Fig. 2, *b-c*). Surrounded by the larger chromosomes, a pair of

quite small ones is always found in the central space of the equatorial plate, showing conspicuously in striking contrast to the others (*z z* in Fig. 2, *b-c*). These two chromosomes seem to constitute a homologous pair in having equal size, very weak affinity for stains and a more or less diffuse outline. The remaining chromosomes,

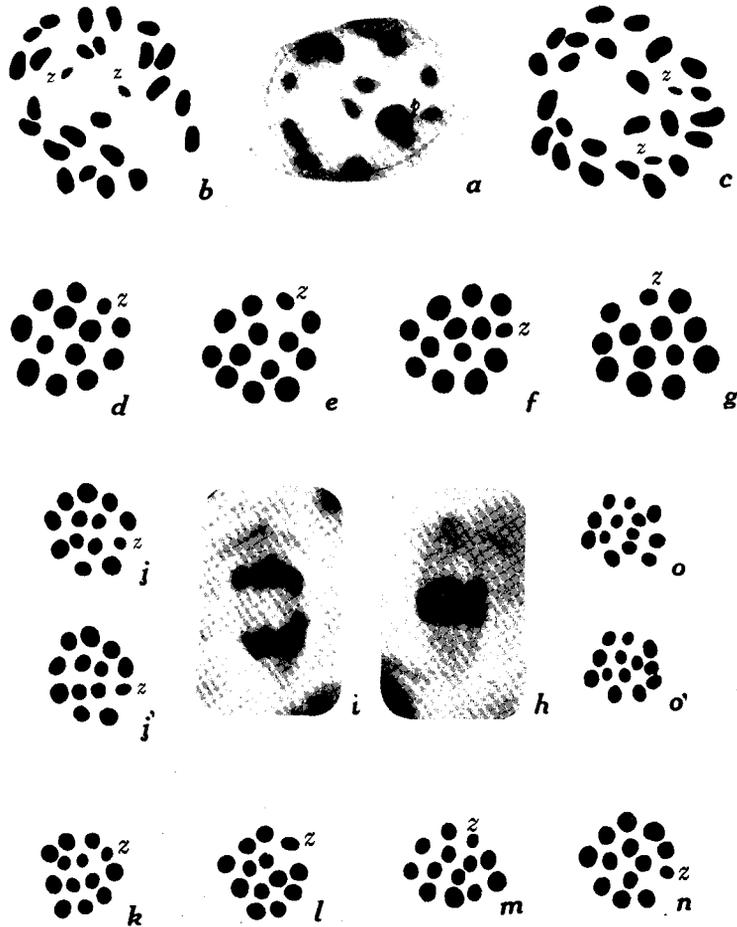


Fig. 2. *a*, spermatogonium in prophase stage. *b-c*, spermatogonial metaphases, 26 chromosomes. *d-g*, primary spermatocyte metaphases, 13 chromosomes. *h*, side view of primary spermatocyte anaphase. *i*, side view of primary spermatocyte anaphase. *j-j'*, sister chromosome groups in anaphase of primary spermatocyte. *k-n*, secondary spermatocyte metaphases, 13 chromosomes. *o-o'*, sister chromosome groups in anaphase of secondary spermatocyte. *b-g*, and *j-o*;  $\times 4200$ . *a* and *h-i*, photomicrographs;  $\times 2700$ .

however, are all compact rod-shaped, somewhat curved and slightly pointed at one end, and do not show any remarkable size-difference from one another.

The spermatogonium contains twenty six chromosomes, one more than the female cell, constituting an even number. When the supposed homologous pairs of chromosomes are checked out by comparison of size and shape, one obtains thirteen pairs of equal size without there being either a solitary unmated chromosome or a pair of unequal size.

*The spermatocyte.*—The primary spermatocyte shows thirteen chromosomes in metaphase with extreme distinctness (Fig. 2, *d-g*). They are nearly round in outline in the polar view. Of these chromosomes, the one that always takes its position at the periphery of the equatorial plate, is distinctly small in size (*z* in Fig. 2, *d-g*). It is probable that this is descended from the two small chromosomes found in the spermatogonium.

All of the thirteen chromosomes are to be considered as bivalent, as expected from the fact that in a spermatogonium thirteen pairs of homologous chromosomes are discovered. This is proved further, when examination is made of the sister sets of the anaphase chromosomes (Fig. 2, *j* and *j'*), in each of which thirteen chromosomes correspond exactly to each other pairwise. Neither precession nor succession in the dividing process takes place in any chromosome in this division (Fig. 2, *h-i*).

The symmetrical distribution of the chromosomes in the primary spermatocyte division gives rise to only one kind of secondary spermatocyte, so far as the chromosome garniture is concerned. The metaphase plate of the secondary spermatocyte invariably contains thirteen chromosomes (Fig. 2, *k-n*). They are quite analogous in shape and arrangement to those of the primary spermatocyte, though reduced in size. Also a small chromosome is always seen situated at the periphery in every equatorial plate (*z* in Fig. 2, *k-n*). In the ensuing division all the chromosomes separate into equal halves. In polar view of the late anaphase, when both sister groups of chromo-

somes are visible in a single section by changing the foci, a quite symmetrical distribution of all chromosomes can plainly be demonstrated (Fig. 2, *o* and *o'*).

Hence, the present observation did not reveal, at least, the occurrence of an unpaired heterochromosome or an unequal pair of idiochromosomes.

#### *The sexual difference of chromosomes*

From the foregoing descriptions it is evident that in the present material there are two different garnitures of chromosomes according to sex. The female cell has twenty five chromosomes, representing the odd number, and the smallest one is evidently devoid of a mate (*z* in Fig. 1, *a-f*).

The corresponding chromosome is, on the contrary, in coupled state in the spermatogonium, in which the number of chromosomes is even (*z z* in Fig. 2, *b-c*). These two chromosomes constitute, in the primary spermatocyte, one bivalent (*z* in Fig. 2, *d-g*), which distributes equally in the resulting secondary spermatocyte by separating into its component halves (*z* in Fig. 2, *j-j'*; *k-n*). The remaining pairs of larger chromosomes (twelve pairs), however, show no difference between the two sexes in number, size or shape. From these interpretations, therefore, it is certain that the small chromosome '*z*' produces the sexual difference in the chromosome constitution. In other words, a garniture composed of twenty four chromosomes plus two *z*'s represents the male, while that of twenty four plus one *z*, the female. Therefore, the chromosome '*z*' is nothing but the sex chromosome. Its particular nature in staining reaction as seen in the spermatogonial metaphase and its peripheral position in the equatorial arrangement in the spermatocytes of two kinds will serve sufficiently for its identification. It is thus proved that the female is heterogametic in consequence of the solitary condition of the smallest chromosome, while the male is homogametic on account of the coupled state of the corresponding one; so that the sex chromosome formula is ZO in the female and ZZ in the male.

A quite similar condition of the sex chromosome has been reported in *Limnophilus decipiens*, a species of Trichoptera, by KLINGSTEDT (1931) who studied chromosome history extensively in the germ cells, not only in the male but also in the female. According to him, *Limnophilus decipiens* possesses twenty chromosomes in the spermatogonium and nineteen in the oogonium. As in the present species, the sex chromosome is identified as the smallest; it is paired in the male cell and unpaired in the female. He observed the chromosomes in the maturation divisions in both sexes and actually ascertained the production of two different kinds of ova, with or without the smallest chromosome in contrast to the sperm cell.

On this occasion, the authors wish to deal briefly with the problem of heteropycnosis of the sex chromosome during the meiotic stages in spermatogenesis. The occurrence of a heteropycnotic karyosome in the auxocyte is repeatedly reported by NAKAMURA (1928,

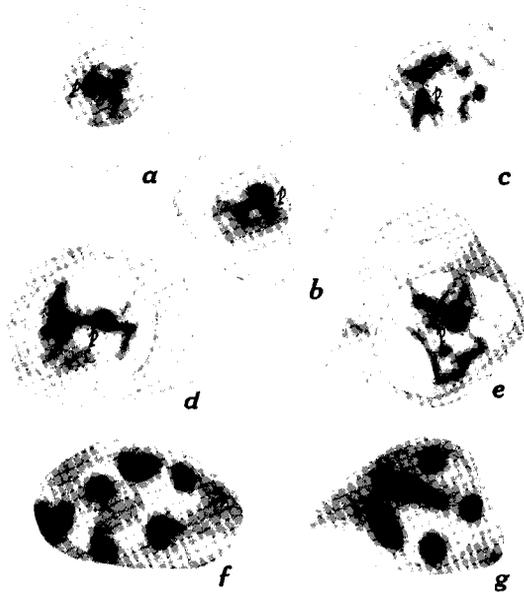


Fig. 3. Photomicrographs.  $\times 2700$ .

*a-b*, nuclei of ultimate spermatogonium. *c*, nucleus in leptotene stage. *d-e*, nuclei in pachytene stage. *f-g*, nuclei in diakinesis.

1932, 1933) in the spermatogenesis of some reptiles, in which the male is homogametic in respect to the sex chromosome. In the present material, in which the condition of the sex chromosome is quite analogous to the reptiles, the evidence is quite different. The nucleus of the ultimate spermatogonium contains a round and intensely stained body ( $p$  in Fig. 3,  $a-b$ ). This body remains unaltered in the nucleus through the earlier stages of the growing period ( $p$  in Fig. 3,  $c-e$ ). With the completion of the tetrads in diakinesis, however, it is completely lost from our vision (Fig. 3,  $f-g$ ). Judging from these evidences, therefore, this body seems to be a plasmosome nucleolus and has nothing to do with the chromosome. It is thus, of course, not the sex chromosome. Similar results have been obtained in *Limnophilus decipiens* and *L. lunatus* by the careful study of KLINGSTEDT (1928, 1931).

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