Studies of sexuality and morphology in the gypsy moth, *Lymantria dispar* L.

II. On the chromosomes of F_1 -hybrids between the weak and strong races.

Kazuo SAITOH

Biological Institute, Hirosaki University.

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It has been well known that gypsy moths comprise three sex-races of different strength. The chromosomes of these sex-races have been studied by several investigators, establishing that there is a uniformity in regard to the number of chromosomes in the three races (Goldschmidt 1917, 1920, 1932, 1934, Makino and Yosida 1945, Saitoh 1956a, b, Seiler 1914). However, little attention has been given to examining the chromosomes of the inter-racial hybrids of gypsy moths, in spite of the extensive morphological studies of their intersex characters. The present paper deals with some results of the observations of the chromosomes of F_1 -hybrids between the weak and strong races, with particular concern to the morphology of the primary spermatocyte chromosomes.

Before going further, the author expresses his cordial thanks to Professor Sajiro Makino, Hokkaido University, for his keen criticism of the data presented in this paper. The author's heartfelt thanks are due to Professor J. Seiler, Eidgenössische Technische Hochschule, Zürich, for his valuable information and also to Professor Hajime Uchida for much aid in various way.

Material and Method: Inter-racial crosses were made in the combinations listed in Table 1. The strong races used were collected from the cities of Aomori, Hirosaki, and Niigata, and from Noheji-machi, Aomori Pref., respectively. The Sapporo-specimens were used exclusively as a typical weak race for the cross. The strong-weak combination produced normal males and females, while the weak-strong combination yielded males alone due to sex-reversal of the genetic females.

In order to make a morphological comparison between the present data and those presented by Goldschmidt (1932), the same technical procedure was adopted in the study of the chromosomes: the Dubosq-Heidenhain procedure was employed on the one hand, and Allen-Bouin's fluid was applied as the fixative on the other hand (Table 1). The fixed materials were prepared following the usual paraffin method, the thickness of sections being 8-10 micra. The accurate chromosome number was studied in the right and left testes of mature Sapporo-Noheji hybrids by the squash method with acetic dahlia (Table 1). As the control, the primary and secondary spermatocyte chromosomes of the parent races were studied under the same conditions of fixation and staining as applied to the hybrids.

Table 1. Data for the material used.

P (♀ × ♂)	Fixative	Stain	No. of ind. stud.
Hirosaki × Sapporo	A.-B.	H. h.	24
Aomori × Sapporo	D.	H. h.	41
Sapporo × Hirosaki	A.-B.	H. h.	33
Sapporo × Niigata	A.-B.	H. h.	53
Sapporo × Aomori	D.	H. h.	64
Sapporo × Noheji	Squash	Acetic dahlia	110

A.-B.: Allen-Bouin's fluid. D.: Dubosq's mixture.
H. h.: Heidenhain's iron hematoxylin and light green.

Figs. 1-20. Chromosomes of inter-racial hybrids of *Lymantria dispar* L., $n=31$ in each. 1-10. Primary spermatocytes, $\times$ ca. 2200. 11-20. Secondary spermatocytes, $\times$ ca. 2000. 1, 2, 11, 12: (Hirosaki $\times$ Sapporo), 3, 4, 13, 14: (Aomori $\times$ Sapporo), 5, 6, 15, 16: (Sapporo $\times$ Hirosaki), 7, 8, 17, 18: (Sapporo $\times$ Niigata), 9, 10, 19, 20: (Sapporo $\times$ Aomori).

Results

Careful examinations of the chromosomes allow the general conclusion that in the fixed material here observed, no morphological difference of chromosomes was produced by the different kinds of fixatives used, nor between the hybrids and the control.

i) **Chromosomes of F₁ strong-weak hybrids:** The fixed materials were exclusively studied here. Many primary and secondary spermatocytes in the process of division were available for close investigation. Generally the chromosomes of primary spermatocyte metaphase are round or oval in outline. So far as the present observations are concerned, no remarkable size-difference is found in the bivalents, although Goldschmidt (1932, 1934) has grouped them as large, medium and small ones. They are decidedly 31 in haploid number (Figs. 1-10). The pairing between homologous mates in each bivalent is regularly undergone, each partner being arranged on the same plane, showing a well-formed metaphase plate. The majority of bivalents arrange themselves with their long axes nearly parallel to the axis of spindle, though there are a few exceptions showing an even arrangement. The chromosome number of the secondary spermatocyte was 31. The chromosomes are also of oval or round shape and arranged well-apart from one another (Figs. 11-14). The individual chromosome is apparently smaller in size than that of the primary spermatocyte. The occurrence of multivalent chromosomes was never observed in either the primary or secondary spermatocytes.

ii) **Chromosomes of F₁ weak-strong hybrids:** The chromosomes were studied with both fixed and squashed materials. The histological examination made it evident that the F₁ gonads show a complete sex-reversal of genetic females.

Upon observing the chromosomes, special attention was drawn to the question whether the genetic female, though it is male as a result of sex-reversal, is cytologically distinguishable from the normal male, or not. The general conclusion was drawn through the following investigation that the chromosomes are wholly indistinguishable between the genetic female and the normal male, so far as the morphology and behavior of the chromosomes are dealt with.

After the examination of fixed primary and secondary spermatocytes it is stated that the descriptions for the chromosomes of the strong-weak hybrids are applicable without any change for the present case (Figs. 5-10, 15-20).

In the squash material, a constancy in the chromosome number was also observed in the haploid complement. Morphologically the bivalent chromosomes are nearly identical to those observed in the fixed material, being oval or round in outline (Figs. 21-24). In the polar view, it is impossible to classify the bivalent chromosomes into three types according to their visible sizes, contrary to the view of Goldschmidt (1932, 1934). Occasionally some variations in size are observable; however, it may probably be the result of uneven pressure applied for squashing the material. The profile view of the spindle shows that the conjugation between homologous mate has taken place in a regular manner.

Figs. 21-26. Chromosomes of inter-racial hybrids of *Lymantria dispar* L., subjected to the squash method with acetic dahlia. $\times$ ca. 1800. 21,22: Primary spermatocytes, $n=31$ in each. 23,24: Secondary spermatocytes, $n=31$ in each. 25, 26: Probably multiploid spermatocytes, 62 chromosomes in each.

Fortunately two cells in which the diploid number of 62 was clearly determined were observed in the left testis of an individual, No. 7 (Figs. 25, 26). At a glance they bear a resemblance to the spermatogonial chromosomes in appearance. From the comparison of cell size between the spermatogonia and spermatocytes, however, it seems probable that they may be primary spermatocytes of a multiploid complex.

Remarks

Suomalainen (1953) has stated as follows: in males of the Lepidoptera the long axis of each bivalent is parallel with the axis of the spindle, whereas in females bivalents lie horizontally on the equatorial plate. In the present material, however, the male hybrids show bivalents which lie horizontally on the equatorial plate. The same situation was found to occur in males of the normal sex-races (Makino and Yosida 1945).

In cytological studies of the geographic races of the gypsy moth, Goldchmidt (1932, 1934) pointed out a racial difference of chromosomes indicating that the amount of chromatin is in an inverse proportion to the strength of sex-races. The comparative study of chromosomes of the geographic races performed by Makino and Yosida (1945) reached the conclusion that the chromosomes are fairly identical in their general appearance, especially in regard to the visible size of bivalent chromosomes, without finding any essential morphological difference in the chromosomes among the different sex-races.

Therefore, it may be safely said that there are no essential differences in the morphological features of the chromosomes of the gypsy moth between the male hybrids and the males of the normal sex-races.

It has been reported that hybrid moths, whose parents have the same number of chromosomes, often show a considerable disturbance in their chromosome numbers (Federley 1928, 1943, 1949, Seiler 1949, Seiler und Puchta 1956). But some authors have found no such abnormality (Kawaguchi and Yosida 1953). It is probable that in hybrids chromosomal inaffinities are primarily the cause for such a disturbance. There is a constancy of the chromosomes both in number and morphology in the inter-racial hybrids of gypsy moths here under study. This may be an indication that the pairing of the chromosomes has taken place in a regular manner during meiosis in the inter-racial hybrids.

It has been stated further that in some hybrid moths the formation of the metaphase plate is often disturbed, resulting in an irregular arrangement of the chromosomes on the equatorial plate (Federley 1949, Seiler 1949). No such irregularity has been known to occur in the *Lymantria*-hybrids here under consideration.

Summary

In F_1 strong-weak and weak-strong hybrids of the gypsy moth the metaphase chromosomes were studied in the primary and secondary spermatocytes, with special regard to the size and behavior of chromosomes. Haploid chromosomes, 31 in number, show the usual circular arrangement at metaphase. They are oval or round in outline without showing any remarkable size-difference, and quite regular in meiotic pairing.

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