Studies of Sexuality and Morphology in the Gypsy Moth, *Lymantria dispar* L. I. On the Gonads of a Bisexual Larva Spontaneously Occurring in the Gifu Race

By

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(*With 22 Text-figures*)

The gypsy moth, *Lymantria dispar* L. shows a remarkable sexual dimorphism, but the occurrence of abnormal individuals provided with bisexual body patterns has hitherto attracted much attention of biologists. They include gynandromorphs and intersexes. Since a gynandromorphic specimen of the gypsy moth was first described by Schäffer (1761), there has been confusion between gynandromorphism and intersexuality (Kosminsky '24, '25). On the basis of his extensive series of studies on sexuality of *Lymantria* inquiring into genetics and physiology of sex, Goldschmidt ('34) has established a marked distinction between gynandromorphism and intersexuality. Three gynandromorphs and one rare case of hereditary gynandromorphism have been recorded by Goldschmidt & Machida ('22) and Goldschmidt ('23, '34), without detailed description of their cytological features. Kopeć ('11) has described a gynandrous transplant in the gypsy moth with some attention to its morphology. In the course of studies of gonadal transplantation with gypsy moths, the present author has recently chanced to find a larval specimen bearing an accessory bisexual gonad, which permitted morphological and cytological investigations in some detail as given in the following.

Before going further, the present author wishes to tender his cordial thanks to Professor Sajiro Makino of Hokkaido University for his keen interest in the problem. The author's heartfelt thanks are also due to Professor Hajime Uchida for much aid extended to him.

Material and method

One egg-batch, pasted on the bark of a tree, was collected in March, 1954 in Gifu City; many larvae hatched out from it in the author's laboratory in May. A specimen carrying an accessory bisexual gonad was found among these laboratory-reared final instar larvae. As controls, some of the larvae of both sexes taken from the same brood were subjected to histological comparison of the gonads. The sections of gonads were prepared following the usual paraffin method, after fixation with Allen-Bouin's solution, and stained with Heidenhain's iron-haematoxylin and light-green.

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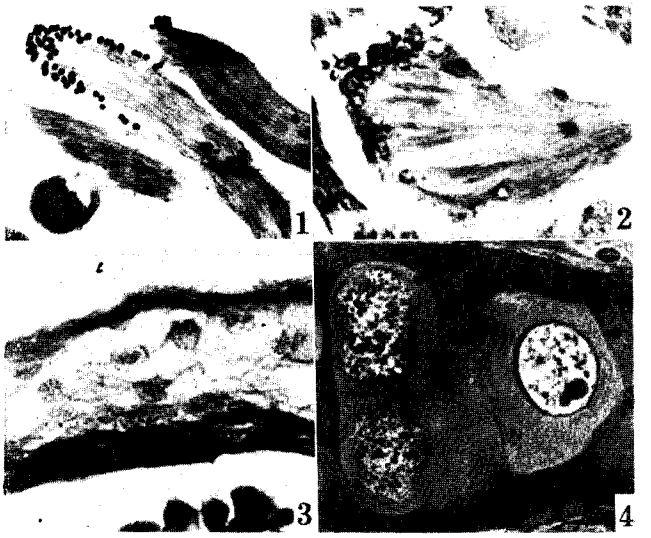
Observations

(1) Morphological aspects of the gonads in normal larvae

(i) *Testis* (Figs. 1-3) : The male larva carries a pair of testes of reniform shape and similar size, each of them being composed of four chambers which contain many bundles of eupyrene spermatozoa. Apyrene spermatozoa happen to occur intermingled with them. A number of morula-like cysts of spermatogonia are distributed in the peripheral part of the chambers. Cysts of primary spermatocytes, blastula-like in appearance, are present along the spermatogonia. To the hilum-side of the testis is attached a sepal-like body which connects directly with the base of each chamber.



Figs. 1-4. Photomicrographs, showing some structures of the testis and ovary of the control larvae. × 400. 1 : Grown spermatids. 2 : Spermatids with elongating tail-parts and nuclei in process of transformation. 3 : Testis-covering. 4 : Egg chamber encircled with follicle cells. Egg and nurse cells are easily distinguishable, and nucleoli are visible in the egg nucleus.

(ii) *Ovary* (Fig. 4) : The female larva bears a pair of ovaries which are of similar size and shape. The ovary contains four bent tubes. The sheath of ovarian tubes consists of one-layered follicle cells. There are two tissue-layers surrounding the sheath of the ovarian tubes. Egg and nurse cells are distinguishable, with the exception of a group of young cells which occupy a proximal part of the tube. The transversal septa of follicle cells develop in each tube. The tube is thus divided into many chambers, each of which contains one egg cell and some nurse cells.

(2) Morphology of the bisexual larva

(i) *External character* : So far as the morphological examination is concerned,

the bisexual larva here under consideration was found to be a male in its external character. There is no evidence for a mosaicism in regard to the bodily color in the present specimen, in contrast to the mosaic specimen described by Goldschmidt ('23).

(ii) *Morphology of gonads*: The specimen under study bears three well-separated gonads, each being different in size (Fig. 5). Their external coverings are deep orange in color, as seen in mature male larvae. The right gonad, large in size, is a testis; it is reniform and is normal in appearance. The left gonad consists of two different parts: one is a medium-sized testicular body and the other is an accessory bisexual gonad, rather small in size. The former is elliptical in form, while the latter is spindle-shaped. The latter is placed on the former, and therefore the ventral view of the spindle-shaped gonad remains unknown. Closer examination reveals no slightest structural connection between the two. The right testicular body and the left double gonad are situated in the 5th abdominal segment, as was also found in the normal larva.

(iii) *The internal morphology of gonads*: The histological structure of the gonads is described for three parts as follows:

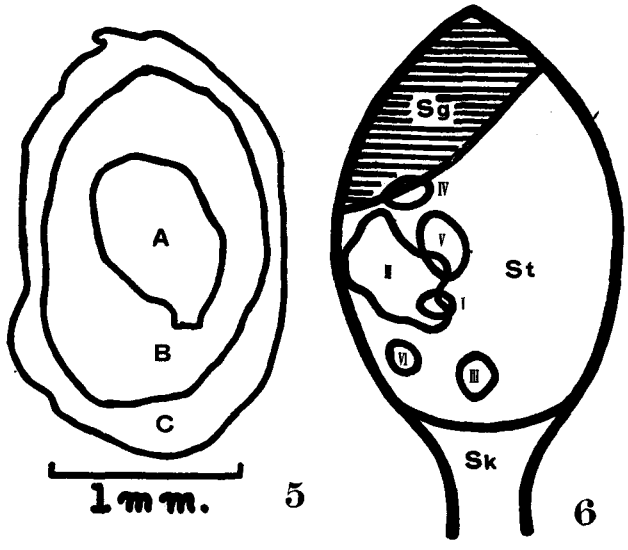


Fig. 5. Comparison of size in horizontal section of three gonads of the present larva, camera-lucida drawings. A: Bisexual gonad. B: Left testis. C: Right testis.

Fig. 6. Schematic diagram projected from ventral side, showing structures of the bisexual gonad. Sg: Spermatogonial region. St: Spermatid-region. Sk: Stalk-like structure. I-VI: Female cells.

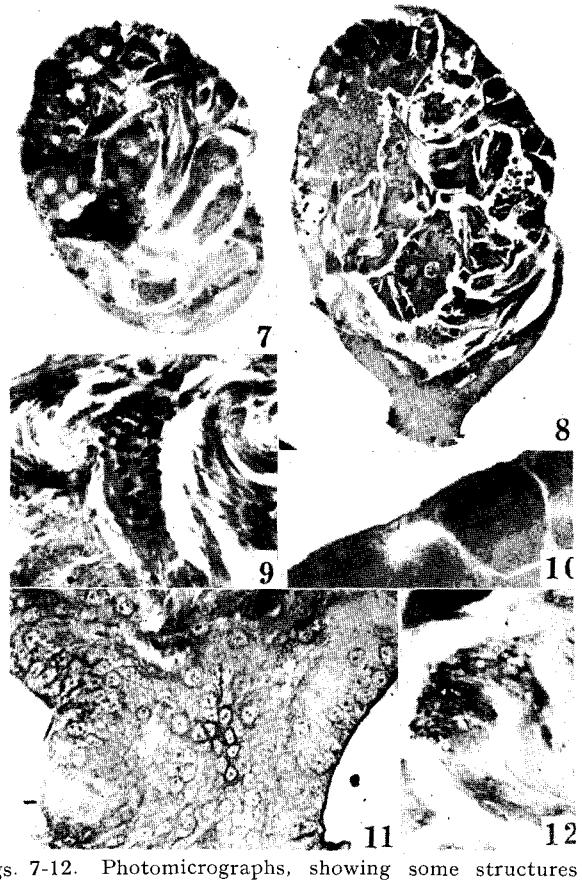
Right testis: This shows an ordinary testicular structure; the inner septa are complete, resulting in the formation of four testicular chambers. Many bundles of eupyrene spermatozoa, a number of apyrene spermatozoa, some spermatids in process of metamorphosis, and a few resting and dividing secondary spermatocytes are present in the chamber. A number of morula-like cysts of spermatogonia and some blastula-like cysts of primary spermatocytes are observed at the anterior periphery of each chamber.

Left testis: This shows also an ordinary testicular structure; it is provided with a single septum, forming two compartments. As was observed in the right testis, there are many bundles of eupyrene spermatozoa, a number of spermatids and a few dividing secondary spermatocytes in those two compartments. A number of morula-like cysts of spermatogonia and blastula-like cysts of primary spermatocytes are observable at the anterior periphery of the compartments. The external coverings and the sepal-like bodies of the right and left testes are regular in structure.

Accessory bisexual gonad: The external covering of the accessory gonad is rather thinner than that of the testicular bodies (Fig. 10). A stalk-like structure is found at the posterior end of this gonad (Figs. 6, 8). It is identical in histological structure with the sepal-like body of the testis, as well as with the stalk of the ovarian tube, though it bears resemblance to the former in appearance (Fig. 11). This gonad shows no septum (Figs. 6, 7, 8), and the single gonadal compartment exhibits two distinctions in respect to the distribution of germ-cells. There are many spermatogonia and primary spermatocytes in the anterior part, while many spermatids together with female cells are observable in the posterior part.

The younger spermatogonia are located at the periphery near the apex of the chamber forming morula-like cysts. A few masses of degenerating cells with pycnotic nuclei are observable among spermatogonial cysts, and degenerating cell-substances which may be derivatives of spermatids happen to occur regionally. A few cysts of primary spermatocytes are found in the boundary part. Spermioteleosis seems to be more delayed in this gonad than in the right and left testes, since no spermatozoa are produced and spermatids are generally in process of metamorphosis (Figs. 9, 12). The tail-parts of spermatids tend to come together in an irregular fashion. It is supposed that most of them may develop into apyrene spermatozoa (Fig. 13). Some grown spermatids are also seen (Fig. 9). From the above evidence the conclusion may be possible that spermatogensis is taking place in almost regular manner in this bisexual gonad.

Remarkable is the fact that the accessory gonad contains forty female cells which are distinguishable from male germ-cells in appearance. They occur in six groups (Groups I-VI), and are arranged from ventral to dorsal in the following order: I, II, III, IV, V, VI, while from anterior to posterior thus: IV, II, V, I, VI, III. Four of them, Groups I, II, IV, and VI, are distributed along the periphery of the right half of the gonad and the remaining two, Groups III and V, lie slightly inside them. The distribution of the six groups is thus limited nearly to the right half of the whole gonad (Fig. 6). Each group contains certain



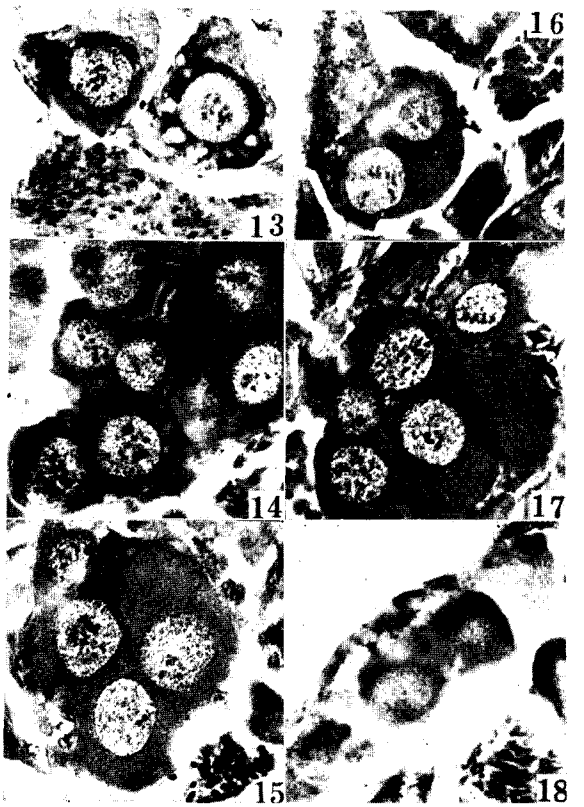
Figs. 7-12. Photomicrographs, showing some structures of the bisexual gonad. 7 : Whole. Parts of three subgroups of Group II are seen. × 100. 8 : Same. A part of Group III is seen. × 100. 9 : Grown spermatids, corresponding to those shown in Fig. 1. × 400. 10 : Thin external covering and morula-like cysts of spermatogonia. × 400. 11 : Stalk-like structure attached to the posterior end of the gonad. × 400. 12 : Spermatids in metamorphic process, corresponding to those of Fig. 2. × 400.

Table 1.

Group of female cells	No. of female cells included in each group	Size of the largest nucleus in each group ($7=20\mu$)
I	2	9 × 9
II	18 = 2 + 2 + 7 + 3 + 4	12 × 10
III	5	10 × 9
IV	4	11 × 10
V	9	11 × 10
VI	2	7 × 6

numbers of female cells as shown in Table 1.

Group I is located at the ventral periphery and Group VI (Fig. 18) at the dorsal. The cells of Group I show vacuoles of various sizes in their cytoplasm (Fig. 13). Group II contains the largest number of female cells, and shows inward elongation from the periphery. It is subdivided into five subgroups (Fig. 14). Group III contains five cells (Fig. 15). Group IV is located at the periphery near the boundary (Fig. 16), and Group V is situated inside it with elongation towards the base of the gonad. One of the cells of Group IV shows four vacuoles

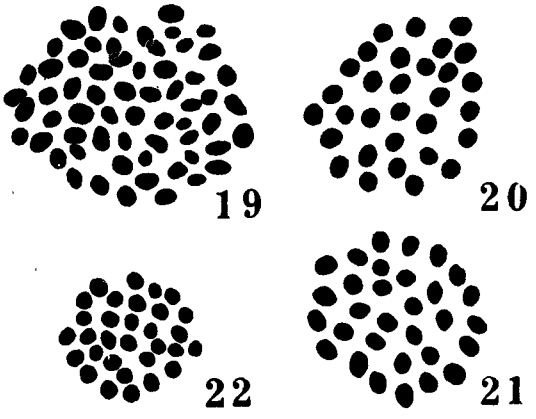


Figs. 13-18. Photomicrographs, showing parts of each group of the female cells. × 400. 13 : Group I. Vacuolar cavities are visible in the cytoplasm. Spermatids to develop into apyrene spermatozoa are seen also. 14 : Group II. 15 : Group III. Heads of grown spermatids are seen at the lower right corner of the photo. 16 : Group IV. Parts of Group V are seen at the lower right corner of the photo. 17 : Group V. 18 : Group VI. Take note of the external covering of the gonad.

in its nucleus. Group V is a mass of nine cells, and assumes as a whole a match-box-like configuration (Fig. 17).

It is remarkable that the absence of follicle cells is common to all groups. The nuclei of these female cells are nearly round or elliptical in outline. Their nuclear contents are generally compact granular substances stained with haematoxylin and therefore the nucleoli are usually indistinguishable. As compared with the egg- and nurse-cells of the control specimens, these female cells of this bisexual gonad show an appearance simulating the nurse cell.

(iv) *Chromosomes*: The diploid chromosomes were observed in three metaphase plates of spermatogonial division occurring in the right testis. They are all 62 in number (Fig. 18), being in agreement with the reported number (Makino & Yosida '45, Seiler '14). The bivalent complexes were examined in some dividing primary spermatocytes found in the left testis and the bisexual gonad. The bivalent chromosomes are round or oval in outline, and 31 in number (Figs. 20, 21). Close observations in both polar and side views have revealed that the meiotic conjugation of chromosomes shows no sign of irregularity. So far as the present material is concerned, there is no evidence for the formation of multivalent chromosomes. The chromosomes of the second division were observed in the left testis and the haploid number was ascertained to be 31 (Fig. 22). The haploid number of chromosomes also agrees with the reported number (Goldschmidt '32, '34, Makino & Yosida '45). In the light of the above observations it is evident that the bisexual larva is undoubtedly a diploid individual, since there



Figs. 19-22. Metaphase chromosomes. All camera-lucida drawings. 19: Spermatogonial chromosomes (right testis), 2n=62. × 3000. 20: Primary spermatocyte chromosomes (bisexual gonad), n=31. × 2000. 21: Same (left testis), n=31. × 2000. 22: Secondary spermatocyte chromosomes (left testis), n=31. × 2000.

is cytologically no evidence for triploidy.

Discussion

It has been shown by the extensive studies of Goldschmidt ('34) that the intersexes of the gypsy moth originate from inter-racial crosses, with the exception of the conspicuous production of intersexual males in intra-racial F_1 of the weak race inhabiting only in Donbas district (Goldschmidt '34, Kosminsky et al. '30). As to the derivation of three gynandromorphs of the gypsy moth reported by Goldschmidt & Machida ('22) and Goldschmidt ('23), attention should be paid to the fact that all were obtained in a descendant from inter-racial crosses, and that both the strong and weak races were originally involved in the cross. On the other hand, Kopeć ('11) found a gonadal transplant, gynandrous in structure, in the abdomen of an ovariotomized adult gypsy moth.

There is a question as to whether the bisexual gonad described in the present paper is intersexual or gynandrous. It is important to see that the larva carrying the bisexual gonad is a descendant from the definite Gifu-race. In other words it is of intra-racial inbreeding origin. Considered from the derivation of the present larva, it is most probable that this gonad may be of gynandrous nature. But, from the structural view-point in comparison with the cases reported by Goldschmidt ('20, '27, '31, '34), Goldschmidt & Poppelbaum ('14), Goldschmidt & Saguchi ('22), Kopeć ('11), and Kosminsky & Golowinskaja ('30), the present bisexual gonad exhibits a similarity in structure to the intersexual gonad rather than to the gynandrous one, slightly suggesting male intersexuality. At present, the author hesitates to make any conclusive statement on the nature of the gonad.

It has been shown that cytologically the present larva bearing the bisexual gonad is of diploid nature, and that there is no evidence for polyploidy.

In the gypsy moth some variations occur in larval testes in relation to number of compartments and to the elementary structures (Saitoh '55). Therefore, it is a problem whether the reduction in number of testicular chambers occurring in the left testis of the bisexual larva above described is due merely to variation or whether it is related at least to the formation of the accessory bisexual gonad. Analysis of this problem will be made in future, after getting more materials available for discussion.

Summary

A larva bearing an accessory bisexual gonad was found in the Gifu-race of the gypsy moth, *Lymantria dispar* L., and its morphological examinations were made at the final instar stage.

1) The larva is externally of male character; it carries a pair of testes of different sizes and an accessory bisexual gonad, small in size.

2) The bisexual gonad shows at its posterior end a stalk-like structure without any septum. While the majority of gonadal contents is male germ-cells, there are present some female cells in the accessory gonad. They form six groups,

each surrounded by spermatids and present the general appearance of nurse cells. The absence of follicle cells is remarkable.

3) Cytologically this bisexual larva seems to be a diploid individual.

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