Drosophila Survey of Hokkaido, XXXIII. Ovarian Development of Drosophila in Relation to Wild Population.

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(*With 4 Text-Figures and 1 Table*)

A number of major studies have been accomplished on the ovary of the drosophilid fly from various standpoints by many investigators (Kambysellis, 1968; King, 1970; Lakovaara *et al.*, 1972; Lumme *et al.*, 1974; and Toda *et al.*, 1975, etc.). In particular, King (1970) summarized ovarian development in *D. melanogaster* from the viewpoints of embryology, histology, physiology and genetics, in great detail. In these reports, only a few analyses were carried out on wild drosophilid assemblages. However, analyses of ovarian development are very useful to clarify the dynamic aspects of wild populations of *Drosophila*, since the generations of each population are revealed by such analyses, and age distribution is an important factor to understand the fluctuation of each drosophilid population (Dobzhansky and Epling, 1944). The ratio of the various age groups in a population determines the reproductive status of the population and indicates how these populations would fluctuate with changes of season.

In addition to this consideration, analyses of ovarian development are needed to clarify the voltinism and life history of each drosophilid species.

Moreover, the postmature stage of the ovary has received little attention (Toda *et al.*, 1975). It is also of interest to study the postmature ovary and its processes for its morphological significance.

For these reasons, there are two main aims: one is adequately to classify ovarian development and the other is to clarify the age distribution of wild drosophilid populations. At first, the continuous changes of ovarian development were observed and divided into four stages based on morphological characteristics. Then the ovarian stages of wild-caught flies were analysed histologically as well as morphologically.

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Materials and Methods

Two species, *Drosophila sordidula* and *D. testacea*, which are common in the suburbs of Sapporo, were used. The methods of culture, and of obtaining paraffin sections, were as follows.

Stocks of the flies were raised and maintained in a standard 180 ml milk bottle on a medium consisting of sugar-agar- Baker's yeast mixtures with propionic acid added as an antiseptic, at a temperature of 18°C with continuous illumination. After adult eclosion, the new-born flies were immediately removed to a culture vial 22 mm in diameter and 50 ml in volume. Thereafter, the females which were with males were periodically anatomized in Waddington's Ringer solution and the ovaries extracted. For microscopic observation, the ovaries were fixed in Bouin's solution and embedded in paraffin. Sections were made seven micra in thickness and stained with Heidenhain's iron-hematoxylin (Fig. 3-IVa) and with Delafield's hematoxylin-eosin (Figs. 3 Ia∼III and IVb). Furthermore, the ovaries of females caught in the wild with retainer traps were cut and stained by the same methods.

Next, all the females collected with traps near Sapporo City from April to the biginning of November were dissected under the binocular microscope and the ovarian stage of each female was ascertained. The results of these dissections kept separate for species, and the population constitution of each drosophilid species was surveyed by using the ratios of flies at each ovarian stage.

Results and Remarks

I. Ovarian development.

Length of time to accomplish the complete development of ovary varies in species. These seemed to be influenced by factors such as genotype, age, temperature, humidity, the degree of adult crowding, photoperiodism and nutrition of the adult female (Herskowitz, 1957; Sang and King, 1961; Wilson *et al.*, 1955, etc.). Lumme and his co-workers (1974) reported that the ovarian development correlated well with insemination. Even though there were some differences in ovarian developmental rate and status among the several drosophilid species observed, four stages of ovarian development could be identified: (1) undeveloped ovary, approximately similar to the stage found in females immediately after adult eclosion, (2) developing ovary, containing growing egg chambers, (3) mature ovary with many completely formed eggs, and (4) postmature ovary with few eggs, or usually, none at all (cf. Fig. 1). The external morphology of each stage examined under a binocular microscope is shown in Figs. 1 and 2. The morphology is very similar in both wild and cultured flies. In addition to the morphological observation, the similarity was also seen histologically under a microscope at every ovarian stage (cf. Fig. 3-I).

On the other hand, King *et al.* (1956) and Cummings and King (1969) classified the development of the egg chamber in the adult *D. melanogaster* ovary into a series of fourteen consecutive stages using criteria such as the size, shape and location of each of the nurse cell, follicle cell, ooplasm and yolk formation. The above

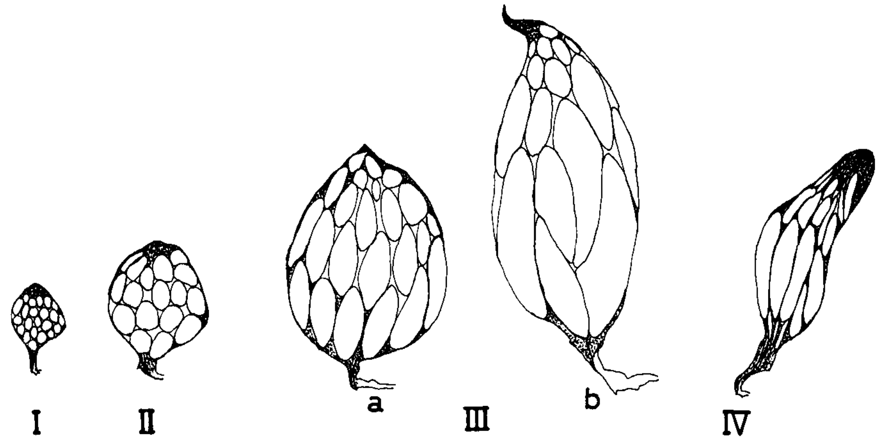


Fig. 1. Process of ovarian development of *D. ezoana*. Age of each stage: I; Stage I, 2 days old. II; Stage II, 7 days old. IIIa; Early stage III, 138 days old, IIIb; late stage III, 154 days old. IV; stage IV, 174 days old.

four stages of overall ovarian development could also be integrated with King's classification of the egg chamber. Since the development of an ovary is a continuous event, the separation of this development was made by relying on multiple morphological criteria. At that time, special attention had to be given to the developmental condition of the most posterior egg chamber.

Stage I. Undeveloped Ovary [Fig. 2-I. The ovary of cultured fly (Ia) was larger than that of wild-caught (Ib), because the former was not fixed.]

Here the ovary is relatively small. The numerous tracheal branches are clearly observed around the ovary. Each ovary consists of a compact group of parallel egg tubes or ovarioles which are relatively uniform in shape and synchronized in their growth rate. The typical value for an inbred strain of *D. sordidula* would be 35±1 ovarioles per ovary. At eclosion the female of *D. sordidula* has a vitellarium consisting of between 3 and 5 chambers – all in the preyolk stage (Fig. 3-I).

The ovary in Stage I has the egg chambers in which yolk formation has not commenced, corresponding to stages 1 to 7 of King's classification.

Stage II. Developing Ovary (Fig. 2-II).

As the ovary develops, the ovarioles increase rapidly in size and volume. Each ovariole contains a chain of developing ova (Fig. 3-II). The degree of asynchronization in the development of the ovarioles is higher than in Stage I.

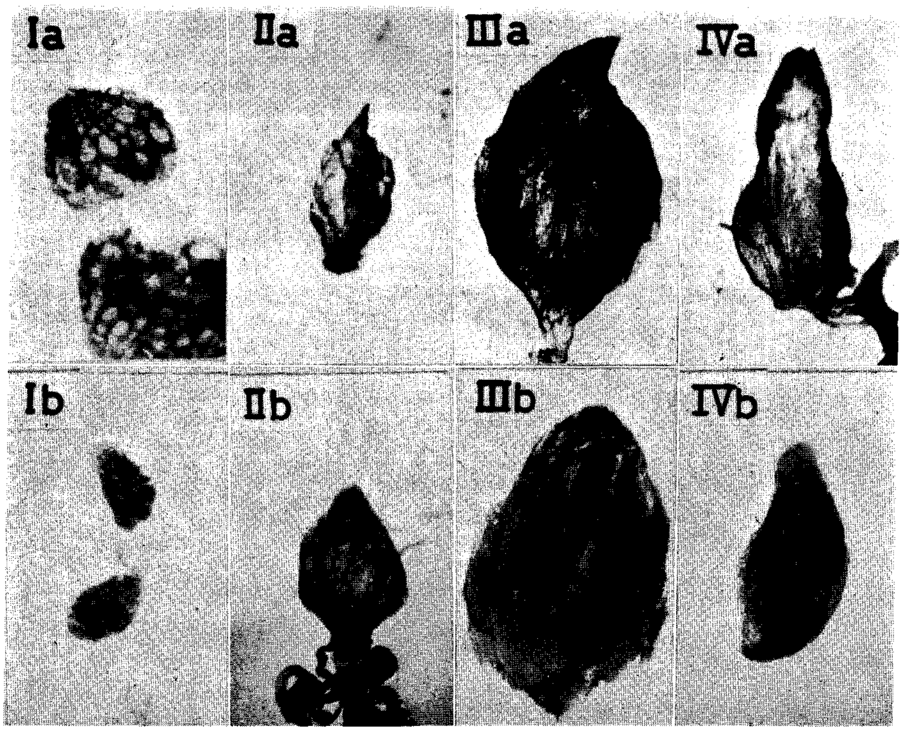


Fig. 2. External morphology of ovaries of *D. testacea*. a: ovaries of cultured flies. b: ovaries of wild-caught flies. (Age of cultured flies. Stage I; 3 days old. Stage II; 11 days old. Stage III; 60 days old. Stage IV; 65 days old.)

Consecutive mitoses occur within the germarium and yolk formation commences in many ovarian chambers within the vitellarium. Subsequently, the ovary grows rapidly. In the latter part of this stage the compartment endochorion is beginning to form between the vitelline membrane and follicle cells (Cummings and King, 1969). In this stage no mature egg is found in the most posterior egg chambers.

The Stage II ovary contains the egg chambers, corresponding to stages 8∼12 of King's classification.

Stage III. Mature Ovary (Fig. 2-III).

Ovaries in early and late Stage III are represented in Fig. 1. They are the largest in volume and size with many mature eggs. The body cavity of the adult female is almost occupied by the reproductive organs. The oocyte has reached its maximum volume and is complete with dorsal chorionic appendages in the most posterior egg chamber of the ovariole. From the middle to the end of this stage, traces caused by the ovulations are clearly shown in the lateral upper oviducts. The epitherial sheath in the vitellarial portion is extremely elongated while there are many eggs in it.

The Stage III ovary contains the egg chambers, corresponding to stages 13～14 of King's classification.

Table 1. Ovarian stages showed by samples of *D. sordidula* from wild population.

Month \ Stage of ovary	I	II	III	IV
June	5/141	14/141	121/141	1/141
September	872/1015	8/1015	18/1015	117/1015

Stage IV. Postmature Ovary (Fig. 2-IV).

a. Description of postmature ovary shape.

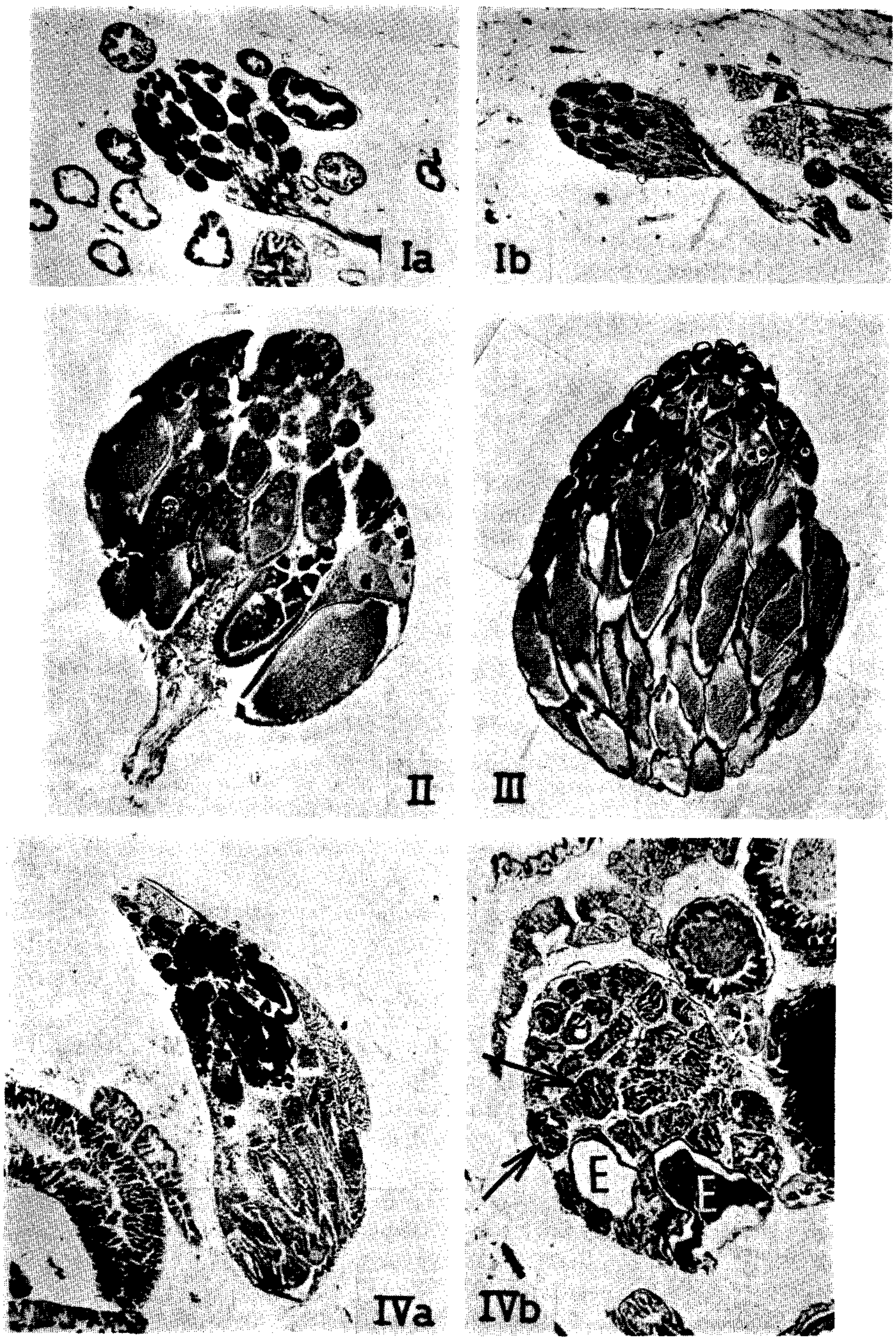
The study on the postmature ovary is very difficult in drosophilid flies. This is due to the fact that few females with postmature ovaries can be found in a wild drosophilid assemblage, being approximately one percent of the total number of drosophilids trapped (Table 1). Even in laborarory culture, most females die with mature eggs.

In *Chrysops bicolor* (Dipt., Tabanidae), a follicular relic is formed in each ovariole after ovulation (Lewis, 1960). This is one of the characteristics which determine the postmature ovary, but not in drosophilid flies (Watanabe and Kamimura, 1971). The postmature ovary of drosophilid flies is characterized by its peculiar elongation, flattened apically, its yellowish brown color and proximately a swollen area containing no or sometimes a few small ovarian ova. The postmature ovary is apt to be confused with the undeveloped one in its appearance. Careful observation enables us to distinguish between them. The former is larger in size and its shape is pear-like. The ovary to which Carson (1948) paid special attention and called "ovaries of this unusual type" might have been postmature ovary from his description.

b. Process of ovarian degeneration.

In our observation, there is no direct process from undeveloped (Stage I) to postmature (Stage IV) without passing the mature period (Stage III), because traces of ovulation were found in the lateral upper oviduct of the postmature ovary without exception. For this reason, it may be said that the postmature ovary is the degenerated one.

In the process of ovarian degeneration, changes in morphology take place in the elongation of the vitellarium portion (Figs. 2-IV and 3-IVa). This elongation is caused by the existence of many mature eggs in Stage III. The basal pedicels of the ovarioles are short when a fully formed egg is present, and temporarily longer after an egg has passed into the oviduct (Miller, 1950). It was also observed that


Ia
Ib
II
III
IVa
E
E
IVb

the pedicel remained elongated in the postmature stage. Therefore, this stage of the ovary formed a peculiar shape (Figs. 1-IV and 2-IV).

It is well known that the consecutive divisions occur within the germarium and the major growth of the oocyte and its accompanying cells occur within the vitellarium. Sectioned preparations of the vitellarial portion showed only a conspicuous network (Fig. 3-IVb). The Stage IV ovary had no or sometimes a few mature eggs. Such facts indicated that a cessation of mitosis of further egg chamber in the germarium also participated in this process. Mitosis had ceased, and after all the mature eggs had passed into the oviduct, no mature egg was found in the ovary. It seems that this postmature ovary was trending to its degenerative process failing the function of its reproductive capacity.

It is difficult to clarify the factor (or factors) which cause ovarian degeneration. In the laboratory culture, most females died with many mature eggs (stage III). For exmaple, one female of *D. sordidula* died 185 days after eclosion within ovarian stage III. In a wild drosophilid assemblage, the ratio of the degenerated ovary was higher in fall than in spring or in summer (cf. Table 1). These facts suggest that most females would die during Stage III in the wild, and two factors, at least, are related to ovarian degeneration. One was unfavorable condition for adult females and the other was the age of the female.

II. Voltinism in wild populations.

As the division of the ovarian developmental process was carried out from the histological and morphological standpoints, the age distribution of each population in every season was known.

As the appearences of flies which had immature ovaries after spring would be due to the newly-hatched flies, the change of generation in the wild population could be roughly clarified. Near Sapporo City, about 20 species were collected abundantly by traps and the number at each ovarian stage was recorded for each species separately. Two types of generation change were seen by the comparison of their generation change throughout the collecting season. One is univoltinism, because flies which had mature ovaries appeared abundantly at only one period (cf. Fig. 4-a). The other is multi-voltinism, because flies which had mature ovaries appeared abundantly 2 or 3 times in a collecting season (cf. Fig. 4-b). Among the 20 species, at least, 2 species, *D. pengi* and *D. moriwakii*, belong to the first type and 15 species, *D. confusa*, *D. bifasciata*, *D. suzukii*, *D. auraria*, *D.*

Fig. 3. Sections of each ovarian stage of *D. sordidula*. Ia: Section of stage I of cultured *D. sordidula* (2 days old). ×100 Ib: Section of stage I of wild-caught *D. sordidula*. ×100 II: Section of stage II of cultured *D. sordidula* (6 days old). ×100 III: Section of stage III of cultured *D. sordidula* (12 days old). ×50 IV: Section of Stage IV of wild-caught *D. sordidula*. a: longitudinal section. ×100 b: cross section of the vitellarium portion. Arrows; conspicuous network of each ovariole. E; mature egg. ×200

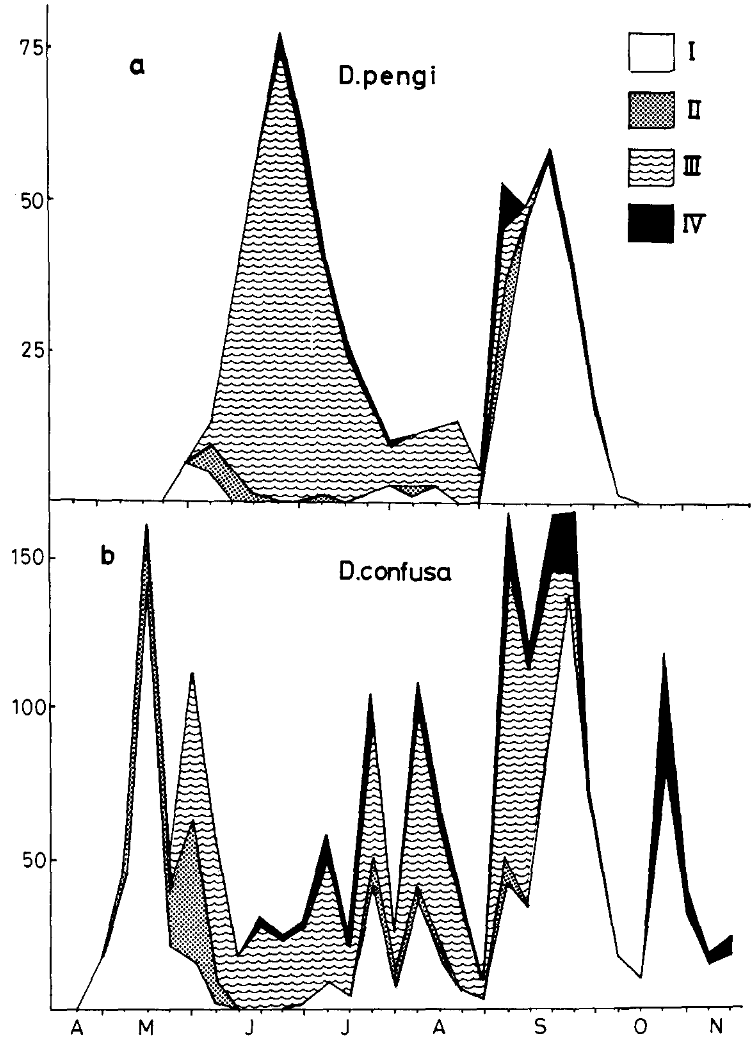


Fig. 4. Age distribution of wild-caught flies, collected at Nopporo, 1975. a; Number of each ovarian stage of *D. pengi*. b; Number of each ovarian stage of *D. confusa*. White; Stage I, Dot; Stage II, Wave; Stage III, Black; Stage IV.

biauraria, *D. testacea*, *D. nigromaculata*, *D. brachynephros*, *D. unispina*, *D. histrio*, *D. immigrans*, *D. ezoana*, *D. sordidula*, *D. pseudosordidula*, and *Amiota variegata* belong to the second type. Two species, *D. okadai and D. neokadai*, were not analysed sufficiently but these species might belong to the uni-voltine group. From these results, most species had a multi-voltine life cycle near Sapporo City, and uni-voltine species were abundant in the *robusta* species group.

Summary

1. Ovarian development was divided into four stages corresponding with the development of the egg chamber of King's classification (1970). Special attention was given to the developmental condition of the most posterior egg chamber.

Stage I. Undeveloped ovary. The whole egg chamber is in the pre-yolk formation stage. King's classification 1 ~ 7.

Stage II. Developing ovary. Yolk formation commences. The comparted endochorion is beginning to form. King's classification 8 ~ 12.

Stage III. Mature ovary. The oocyte is completely sealed by the vitelline membrane and the endochorion is complete. King's classification 13 ~ 14.

Stage IV. Postmature ovary. There were few formed eggs or none, usually. The vitellarium portion remained elongated. The morphological changes were due to the elongation of the vitellarium portion and perhaps the cessation of mitosis in the germarium.

2. There were two types of drosophilid generation change in a year. One was uni-voltinism and the other multi-voltinism. Two species, *D. pengi* and *D. moriwakii*, belong to the first type, and Two species, *D. okadai* and *D. neokadai*, may be uni-voltine too. Fifteen species collected abundantly by traps, near Sapporo City, *D. confusa*, *D. bifasciata*, *D. auraria*, *D. biauraria*, *D. suzukii*, *D. brachynephros*, *D. nigromaculata*, *D. unispina*, *D. testacea*, *D. histrio*, *D. immigrans*, *D. ezoana*, *D. sordidula*, *D. pseudosordidula*, and *A. variegata* belonged to the second type.

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