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Drosophila Survey of Hokkaido, XII. The Salivary Gland Chromosomes of *Drosophila ezoana*
(a Preliminary Report)

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(With 8 Text-figures and 1 Table)

Since the discovery of the genetic implication of the salivary gland chromosomes of *Drosophila*, rather many publications have been devoted to cytological elucidation of many genetic complications, especially in relation to chromosome aberrations, evolutional mechanism of species formation, and so on.

Drosophila virilis is one of the most widely distributed members of the *Drosophilidae* and is well known on account of having been the subject of well-explored cytological and genetical studies. The salivary gland chromosomes of this species from Japan have been investigated by Fujii (1936, 1942) and Makino (1938) ; they consist of five rod-shaped elements and a minute dot-like one.

Recently, *Drosophila ezoana* was described from Hokkaido as a new species of *virilis* group by Takada and Okada (1958). The present paper is to report the results of preliminary observations on the salivary gland chromosomes of *D. ezoana* by way of comparison with those of *D. virilis*.

Here the author wishes to express her sincere gratitude to Professor Sajiro Makino for his keen interest in the subject and kind improvement of the manuscript for publication. Further, the author must express her cordical thanks to Dr. Eizi Momma for important suggestions. Thanks are also due to Mr. Haruo Takada who lent kind help in collecting the material used.

Material and Mehtod : Material for this study was obtained from a strain culture established in our laboratory. The original females of this strain were collected by Takada at above 100 m elevation at the foot of Mt. Raus (altitude 1661 m) on the Shiretoko Peninsula at the eastern extremity of Hokkaido, in August 1958.

The preparations were made as follows : The salivary glands were dissected from mature larvae in Ringer's solution (*Drosophila* formula). The glands were then placed on a slide with a drop of 1 N HCl for about 3 minutes, and stained with orcein solution saturated in 60 per cent acetic acid.

Observations

It was found that the salivary gland chromosomes of *Drosophila ezoana*

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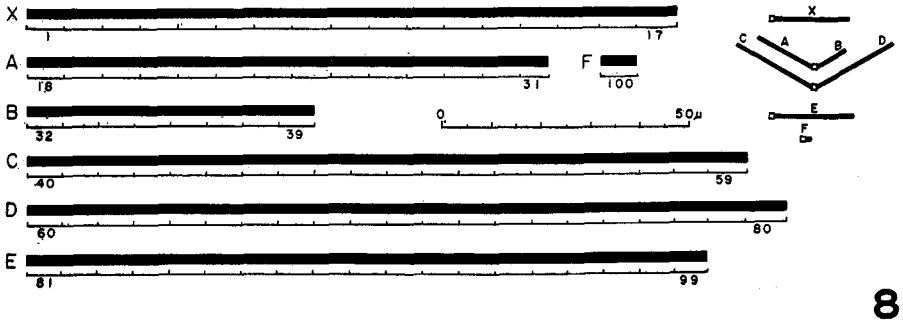
include five long arms, one medium-sized arm and one dot, all of the seven arms being connected by a heterochromatic chromocenter (Fig. 1). The mean length of each arm based on the close analysis of ten cells are as shown in Table 1. A schematic diagram of the salivary chromosomes is shown in Figure 8. It is evident from the figure that the chromosomes are divided into 100 arbitrary sections. The somatic chromosomes in correspondence to the salivary gland chromosomes are shown in the right upper corner of Figure 8. The centromeres are designated in the diagram by blocks. The hundred sections were numbered in the natural order starting from the distal end of the X arm, reaching the base of X, continuing to the distal end of A limb, and after crossing the chromocenter numbering proceeds to the base of B, where the numbers pass the distal end of C, and so on. Thus, the sections of each arm divided here are as shown in Table 1, in connection with their lengths.



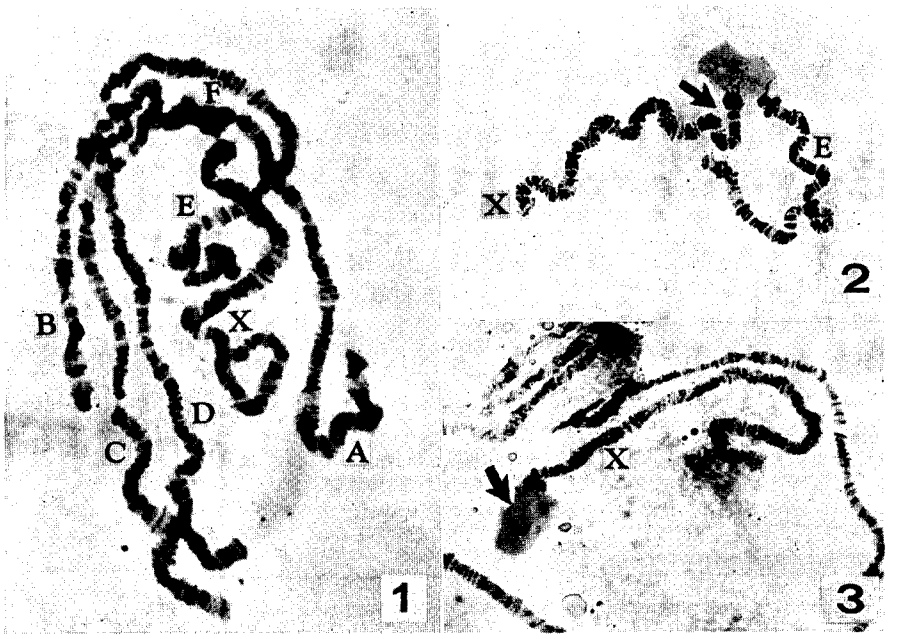
8

Fig. 8. Schematic illustrations showing the orders of seven arms in the salivary gland chromosomes of *D. ezoana*.

Table 1. Lengths of seven elements of the salivary chromosomes of *Drosophila ezoana*

Element	Mean length	Section
X	129	1-17
A	106	18-31
B	58	32-39
C	147	40-59
D	150	60-80
E	137	81-99
F	7	100

The X chromosome was identified by the presence of the nucleolar organizer (Figs. 1, 2, and 3) and by its possession of a "weak point" area on the distal pattern of the chromosome. The mean length of the X chromosome is about 129μ, and it shows sections from 1 to 17. It was observed as a rod-shaped chromosome



Figs. 1–3. Photomicrographs of the salivary chromosomes of *D. ezoana*. 1, seven arms in the salivary chromosome of *D. ezoana* female. ×700. 2, X and E chromosomes. Nucleolar-organization of X chromosome is indicated by arrow. ×400. 3, X chromosome. Nucleolus is indicated by arrow. ×450.

which is a medium-sized strand amongst the seven arms.

The A and B chromosomes were identified by being unequal in length of their two limbs (Figs. 1 and 4). The longer limb is designated as A and the shorter one as B. The mean length of the A chromosome is about 106 μ (sections 18–31), and that of the B chromosome about 58μ (sections 32–39). The B chromosome is about one-half the A chromosome in relative length. The A and B limbs are connected by the chromocenter and form a J-shaped chromosome.

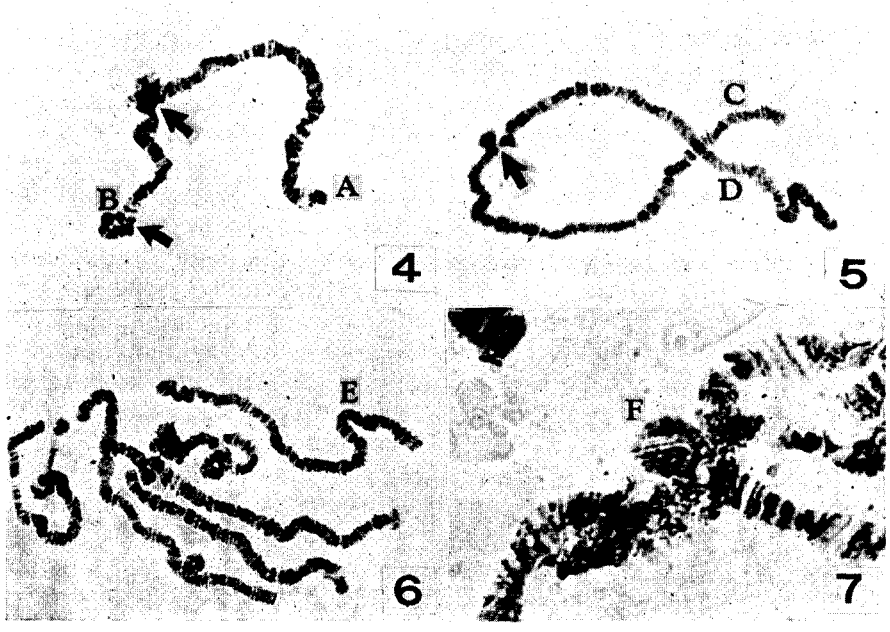
The C chromosome which has a length of about 147μ shows sections from 40 to 59. The D chromosome is about 150μ in length and shows sections from 60 to 80. The C and D limbs form a large V-shaped chromosome (Figs. 1 and 5).

The E chromosome which shows sections from 81 to 99 is about 137μ in length. It is easily recognizable as a rod-shaped formation as shown in Figures 1, 2 and 6.

The F chromosome which is characterized by section 100 is a minute dot-like element, about 7μ in length (Figs. 1 and 7). It is very difficult to detect the F chromosome, because it is extremely small in size, usually included in the chromo-

center.

In addition to the above findings, a small loop was found at the distal part of the B chromosome (Fig. 4). This aberration was found in the samples from a strain cultured about six months in our laboratory. No such configuration has been observed in the material from the first culture generation : detailed analysis of this aberration has now been carried out by the present author.



Figs. 4–7. Photomicrographs of the A, B, C, D, E and F chromosomes of *D. ezoana*. 4, A and B limbs extended out from the chromocenter which is inidcated by arrow. The other arrow shows a small loop on distal position of B chromosome. ×400. 5, C and D limbs with about equal lengths. Both are connected by the chromocenter which is indicaetd by arrow. ×400. 6, E chromosome of rod-shaped. ×400. 7, F chromosome of dot-like. ×900.

Discussion

Drosophila virilis is a member of the world-wide distributed *Drosophila* when has been found to occur in four different zoogeographic realms. On the basis of genetic tests, ten known forms of the *virilis* group were reported by Patterson and Stone (1952) as follows : 1) the most widely distributed member, *virilis*, which probably in native in the Palaearctic and Oriental regions, 2) the closely related forms, *americana*, *texana* and *novamexicana*, occur in North America, 3) the most distantly related North American forms are *montana*, *flavomontana*, *borealis* and *locicola*, while 4) *littoralis* and *imeretensis* are both

European forms.

The somatic chromosomes of *D. virilis* from Japan have been investigated by Chino (1936-1937, 1941), Fujii (1937, 1938), Makino (1940, 1942) and Momma (1954). The karyotype of *D. virilis* was found to show five pairs of rod-shaped chromosomes and a pair of dot-like ones. It has been shown further that the ten known members of this group show five different types of metaphase configurations (Heitz 1934, Patterson, Stone and Griffen 1940, 1942 Stone, Griffen and Patterson 1942, Patterson 1944, Hughes 1939b, Sokolov 1948, Hsu 1952, Ward unpublished). The chromosome complement of the European form *D. littoralis* was found to consist of two pairs of rods, a pair of V's, a pair of J's and a pair of dots (Hsu 1952, Ward unpublished). According to Momma (unpublished data), *D. ezoana* shows a metaphase configuration closely similar to that of *D. littoralis*.

The present observations have revealed that the salivary chromosomes of *D. ezoana* consist of two rod-shaped, a large V-shaped, a medium-sized J-shaped and a dot-like elements. Reported evidence has shown that the salivary chromosomes of *D. virilis* include five rod-shaped and one dot-like elements. It is evident after comparison that the salivary galnd chromosomes of *D. ezoana* are clearly different from those of *D. virilis*. On the other hand, the salivary gland chromosomes of *D. littoralis* of European form were found to comprise six arms and a dot-like element (Ward, unpublished). The salivary chromosomes of *D. ezoana* seem to be similar to those of *D. littoralis* (Ward, unpublished). A comparative analysis of the salivary gland chromosomes of *D. ezoana* and *D. littoralis* is not presented in this paper, since no detailed report of the salivary chromosomes map of *D. littoralis* has been published as yet.

Summary

The salivary gland chromosomes of *Drosophila ezoana*, a new species of the *virilis* species group from Hokkaido were described in this paper. A schematic illustration of the salivary chromosomes was given: the salivary chromosome complement consists of two rod-shaped, a large V-shaped, a medium-sized J-shaped and a dot-like elements. The salivary chromosomes of *D. ezoana* apparently differ from those of *D. virilis*. There is evidence to show that the salivary chromosomes of *D. ezoana* are nearly similar to those of *D. littoralis*.

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