Drosophila Survey of Hokkaido, II. Chromosomes of Seven Wild Species[1)]

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(With 34 Text-figures)

A study of the chromosome pattern in *Drosophila* in relation to the problem of species differentiation has been the matter of repeated investigation by many workers. Up to the present, a number of *Drosophila* species have been karyologically dealt with, and data on this matter are being accumulated. For the magnitude of work carried out on this line, one may refer to the list of the chromosome numbers compiled by Makino (1951).

During recent years, it has been a common practice to examine the chromosomes of *Drosophila* by means of acetocarmine smear preparations of the larval giant nerve cells, or of ovarian germ cells. But, by the application of smear methods certain confusion has frequently arisen because of the production of an irregular metaphase arrangement of chromosomes or distortion of metaphase configuration, resulting in the appearance of sharp constrictions in chromosomes. For this reason it is a common feature the descriptions of *Drosophila* chromosomes that the metaphase configurations are generally illustrated by semidiagrammatic drawings.

Drosophila as a whole furnishes material that is unfavorable to cytological investigation, on account of the samll size of the cells ; particularly, the detailed study of the meiotic features of this group is difficult because of the small size of the gonads together with the rather complicated behavior of chromosomes in meiosis associated with deficiency, duplication, translocation, inversion and some other aberrations. This is the reason why there have been a rather few reliable cytological studies on the group.

The investigations to be described herein have been undertaken at the suggestion and direction of Professor Makino with the purpose to study the chromosome pattern, as well as to examine the meiotic phases in males if possible,

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in many wild forms of *Drosophila* collected in the course of a survey in various localities of Hokkaido. In the present paper there are recorded both mitotic and meiotic chromosomes of seven species so far observed.

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Material and methods

The following descriptions refer to the chromosomes of seven species, viz., *D. alboralis, D. busckii, D. rufa, D. nigromaculata, D. virilis, D, sordidula* and *D.* sp. The flies used for material are all derived from the wild stocks which were obtained by the Drosophila survey in Hokkaido. In every species, both testes and ovaries of newly emerged flies were adopted as material. For fixation the following new fixative was exculusively employed with excellent results.

Saturated aqueous solution of picric acid	75 cc
Formol	25 cc
Acetic acid, glacial	25 cc
Urea	1 g

Following the ordinary paraffin method, sections, 5–7 micra thick, were made, and subjected to Heidenhain's staining method with iron-haematoxylin and light green. The chromosomes were drawn with the aid of a camera lucida at a magnification of approximating 4500 times.

Observations

Subgenus *HIRTODROSOPHILA*

Two species, *D. duncani* and *D. orbospiracula,* were cytologically studied in this subgenus (Wharton 1943). It was found that the male of *D. duncani* has 10 chromosomes, consisting of three paris of V-shaped autosomes, a pair of V-shaped microchromosomes, a V-shaped X and a rod-shaped Y. The male of *D. orbospiracula* has 11 chromosomes which include four pairs of autosomal rods, a pair of small dots and a rod-shaped X with a proximal constriction. The sex-chromosome mechanism of an XO type in this species is a matter of question.

1. *Drosophila alboralis*

This form was recently recorded by Momma and Takada (1954) as a new species belonging to *Hirtodrosophila.*

In polar view of the oogonial metaphase, there were 12 chromosomes arranged radially in the equatorial plate (Fig. 1). From their external configurations, all of them seem to be telomitic in the spindle fibre attachment. The complement showed four pairs of autosomal rods varying slightly in size, a pair of autosomal dots and a pair of elongated X-chromosomes. The spermatogonial complement consisted of 12 chromosomes which contained four pairs of autosomal rods, a pair of dots, an elongated X-element and a rod-shaped Y,

somewhat shorter than the X (Fig. 2).

The haploid set as observed in the primary spermatocyte showed 6 chromosomes which consisted of five large bivalents assuming the form of a heavy rod and a small dot-like one (Figs. 3–4).

Remarks: Referring to the results of Wharton (1943) concerning *D. duncani* and *D. orbospiracula*, it is evident that the chromosome pattern of *D. alboralis* here reported bears much resemblance to that of *D. orbospiracula*, except for the fact that the male sex-chromosome mechanism is of the XY type in the former, while it is of the XO type in the latter.

Subgenus *DORSILOPHA*

2. *Drosophila busckii*

Sirotina (1938) is the first to report in detail the chromosomes of this species using Russian specimens. Following him, Kikkawa and Peng (1938), Wharton (1943) and Krievshenko (1952) contributed important informations on the chromosomes of the same form. Referring to their reports it is apparent that this species presents three different karyotypes; 1) consisting of two pairs of large V's and the X and Y which are rod- or J-shaped (Wharton 1943, Krievshenko 1952), 2) having two pairs of large V's, a J-shaped X and a V-shaped Y (Sirotina 1938), and 3) showing two pairs of large V's, a pair of dot-like ones, and the X and Y which assume a rod-shape (Kikkawa and Peng 1938).

The oogonial metaphase observed in this study showed 6 chromosomes; they were provided with two pairs of large V-shaped autosomes of a submedian nature and a pair of rod-shaped, sometimes saber-shaped, X-chromosomes (Fig. 5). In the spermatogonial metaphase, the sex-chromosome pair was represented by a rod-shaped X with a constriction at its proximity, and a Y of rod-shape (Fig. 6).

The primary spermatocyte metaphase showed two large V-shaped bivalents and an XY-complex in an end-to-end connection (Figs. 7–8).

The secondary spermatocytes showed at metaphase two different plates; one contained two large V-shaped dyads and a rod-shaped Y (Fig. 9), and the other comprised a similar set of autosomal dyads and an X (Fig. 10).

Remarks: Krievshenko (1952) has found that the marked constriction can be observed at a point about one-quarter to one-third from the proximal end of the X-chromosome. According to him this is not a centric constriction, because the chromosome always lies with its proximal end directed towards the center of the equatorial plate. A similar feature was encountered in the material observed by the present author (Figs. 5–6). According to Sirotina (1938) the nerve cells from the larval brain of the male Russian specimens have a metacentric Y-chromosome. Kikkawa and Peng (1938) described a pair of dot-like ones in excess. No such additional elements have been detected in the Hokkaido material.

Further, Krievshenko (1952) has examined the chromosomes of this species coming from America, England, Italy, Japan and Brazil, and failed to demonstrate any dot-like elements as described by Kikkawa and Peng (1938).

To summarize, the general configuration of the chromosomes as observed in the present study falls in agreement with that reported by Wharton (1943) and Krievshenko (1952), either in the number of chromosomes or in their morphological characteristics.

Subgenus *SOPHOPHORA*

melanogaster group

Cytological studies have been made of 12 species of this group ; they are characterized by several different chromosome patterns.

3. *Drosophila rufa*

Kikkawa and Peng (1938) have briefly described the chromosomes of this species, stating that the metaphase complement shows two pairs of autosomal V's, a pair of dot-like ones and a rod-shaped X.

The oogonial chromosomes were found to be 8 in number ; they comprise two pairs of large metacentric autosomal V's of approximately similar size, one pair of dot-like elements and a pair of rod-shaped X-elements (Fig. 11). The length of the X seems to nearly correpsond to that of one arm of the autosomal V-shaped element. The spermatogonial metaphase showed the same set of autosomes in addition to the X- and Y-elements (Fig. 12). The Y bore a small knob-like thickening near the centromere region.

The primary spermatocyte metaphase was found to contain 4 bivalent chromosomes (Fig. 13). They were provided with two large V-shaped elements, a small dumbbell-shaped dot and an XY-complex of thick rod-shape.

The secondary spermatocyte showed again 4 chromosomes at metaphase, comprising two large V's, a small dot- and a rod-like sex-chromosome (Fig. 14).

Remarks : Six different karyotypes have been known in the *melanogaster* group. They are ; 1) 4V's+2D's+R(X)+R or J(Y) for *melanogaster* and some others, 2) 4V's+2R's+R(X)+R(Y) for *montium* I, 3) 4V's+2R's+R(X)+v(Y) for *montium* II, 4) 4V's+2v's+R(X)+v(Y) for *montium* III, 5) 4V's+2v's+V(X)+V or J(Y) for *bipectinata* and *ananassae*, and 6) 4V's+J(X)+R(Y) for *takahashii* II. Referring to the above classification, it is evident that the karyotype of *Drosophila rufa* belongs to type 1. Thus the results of the present observations are similar to those of Kikkawa and Peng (1938), except that the Y-chromosome has a knob-like thickening at its kinetochore region.

Subgenus *DROSOPHILA*

quinaria group

The chromosomes of this group have been studied by previous authors,

and eight different karyotypes have been demonstrated so far.

4. *Drosophila nigromaculata*

This species was described as a new one belonging to *quinaria* group by Kikkawa and Peng in 1938, but there is no reference to the chromosomes of this species in the literature.

Both oogonial and spermatogonial metaphases of the present species showed 12 chromosomes in diploid (Figs. 15–16). They consisted of three pairs of large rod-shaped autosomes, a pair of rod-shaped autosomes of considerably small size, a pair of dot-like ones, and two large rod-shaped sex-chromosomes. The sex-elements are represented by two X's in the oogonium (Fig. 15), while by an X and a Y in the spermatogonium (Fig. 16). Sometimes, the Y-chromosome was found to carry a constriction, though not very distinct (Fig. 16).

There were six bivalent chromosomes in the primary spermatocyte (Figs. 17–18). They are represented by three large and one medium-sized elements assuming a thick rod-shape or lanky ring-shape, one small element of dumbbell-shape, or dot-like, and an XY-complex.

The secondary spermatocyte showed again 6 dyad chromosomes provided with three large- and one medium-sized rods, one dot-like one, and the elongated X- or Y-element (Figs. 19–20).

Remarks: Referring to the literature it is apparent that the karyotype of this species closely resembles that of *D. transversa, D. plustris, D. subplustris, D. occidentalis* (Wharton 1943) and *D. mutandis* (Tan, Hsu and Sheng 1949). Mizuno (1952) described 10 chromosomes represented by small rod-shaped elements for this species. In comparison of his results with those of the present study, it seems highly probable that he made some mistake in identifying the species on which his study was based.

virilis group

The literature shows that the chromosomes of 10 species have been reported. There are five different karyotypes in this group so far as the observations have gone.

5. *Drosophila virilis*

Through the work of Heitz (1934), Makino (1940), Chino (1936, 1937), Fujii (1936, 1942) and many others, the karyotype of this species was found to possess five pairs of rod-shaped chromosomes in addition to a minute pair of dot-like ones.

The chromosome number of *D. virilis* was found to be 12 in the observations of both oogonia (Fig. 21) and spermatogonia (Fig. 22). The diploid set was characterized by five pairs of rod-shaped elements with a slight variation of size together with a pair of minute dot-like ones. Two of the rod elements represent the X-chromosomes in the oogonium, but the X- and Y-elements in the spermatogonium. They are morphologically indistinguishable between the two sexes.

The primary spermatocyte metaphase showed 6 bivalent chromosomes consisting of four large autosomal bivalents, one minute bivalent and a large XY bivalent (Figs. 23–24).

In the secondary spermatocyte there were 6 dyads provided with four large elements, one dot-like one and a large X- or Y-chromosome (Fig. 25).

Remarks: Wharton (1943) has described the following four karyotypes in the female of this group. 1) 10R's+2D's for *virilis* and *novamexicana*, 2) 2V's+6R's+2D's for *texana*, 3) 4V's+2R's+2D's for *americana*, and 4) 8R's+2v's+2D's for *montana*. In every morphological respect, the chromosomes of the present species are nearly identical to type 1 of the above classification.

robusta group

The chromosome configurations have been reported for the following five species: *D. robusta* (Carson and Stalker 1947), *D. colorata* (Wharton 1943), D. *sordidula* (Kikkawa and Peng 1938), *D. cheda* and *D. pullata* (Tan, Hsu and Sheng 1949). They differ in their karyotypes from one another.

6. *Drosophila sordidula*

Both oogonial and spermatogonial metaphases of *Drosophila sordidula* showed 10 chromosomes in diploid (Figs. 26–27). They were found to include a pair of small V-shaped autosomes, two pairs of autosomal rods of different sizes, a pair of dot-like ones, and two large V-shaped sex-chromosomes which are two X's in the oogonium (Fig. 26), but the X- and Y-elements in the spermatogonium (Fig. 27).

There were in the primary spermatocyte two thick rod-shaped autosomal bivalents, one small V-shaped bivalent, a dot-like one, and a large XY-complex (Figs. 28–29).

The chromosomes of the secondary spermatocyte were 5 in number (Fig. 30), comprising two rods, one small V, one dot and a large V-shaped sex-chromosome.

Remarks: The general configuration of the chromosomes of *Drosophila sordidula* observed in this study is nearly identical with that reported by Kikkawa and Peng (1938) for the same species.

7. *Drosophila* sp.

Though the name of this form has not been definitely determined the present species bears a resemblance to *Drosophila robusta* in many characters. A study of the morphological characters is now in progress for identification of the name with the culture stock maintained in this zoological laboratory.

The chromosome number of this species was found to be 12 in both oogonia (Fig. 31) and spermatogonia (Fig. 32). The diploid complement showed five V-shaped various-sized chromosomes of a submedian nature in addition to a pair of very minute dot-like ones. The sex-chromosomes were found to be the two largest

V-shaped elements which were two X's in the oogonium (Fig. 31), and an X and a Y in the spermatogonium (Fig. 32). To discriminate between the X- and Y-chromosomes in the spermatogonial metaphase is very difficult on account of their similar configuration (Fig. 32).

The primary spermatocyte metaphase contained 6 bivalents which were represented by two large rings, two thick rods, a dot-like one, and a large XY-complex (Fig. 32).

The metaphase plate of the secondary spermatocyte disclosed 6 dyads consisting of four V-shaped elements, a dot-like one, and a large V-shaped X, or Y. The dot-like element was frequently found separated prior to the others at the end of metaphase (Fig. 34).

Remarks : So far as the author is aware, there is no species in *Drosophila* which possesses the karyotype as found in this species. It is interesting that this unrecorded species is characterized by such an unique karyotype never described in the literature.

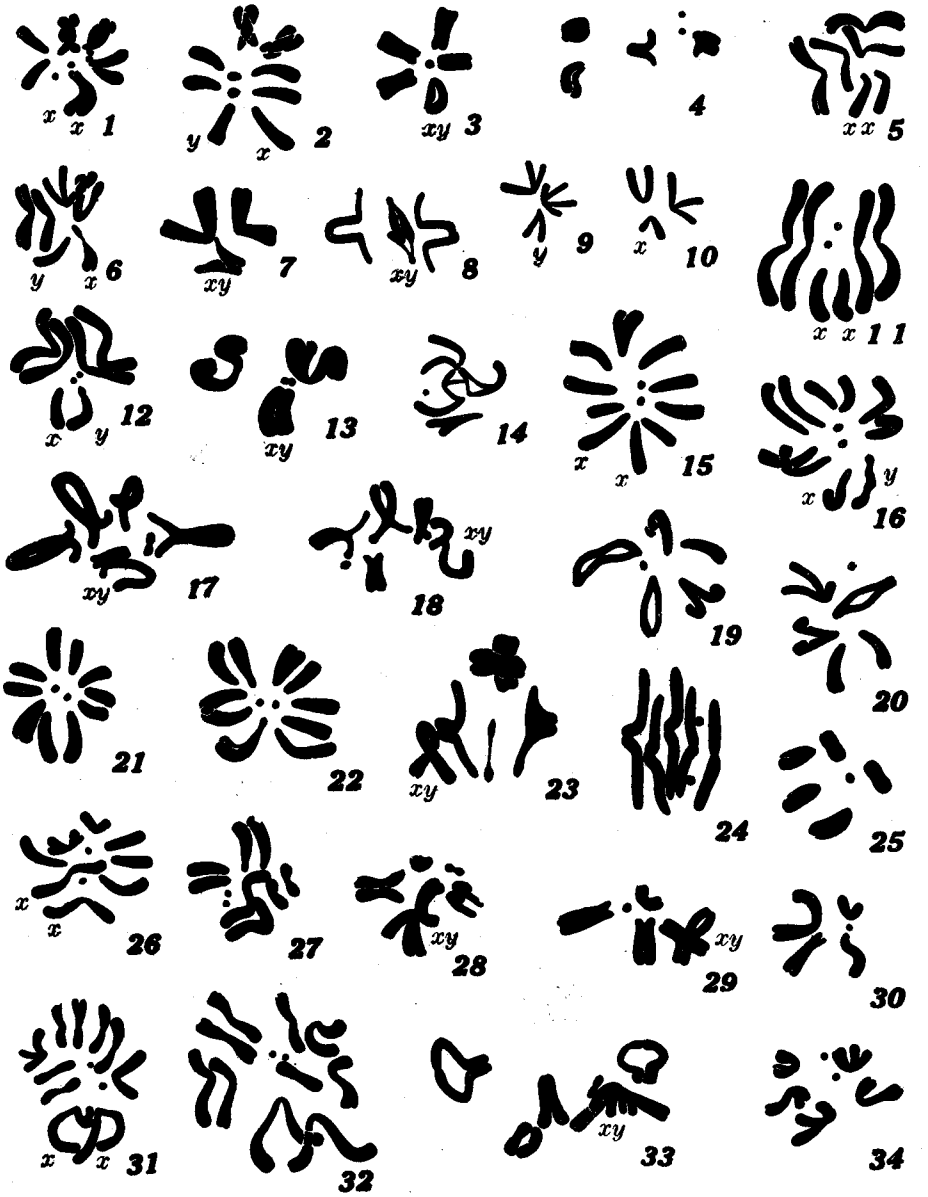
Summary

The chromosomes of seven wild forms of *Drosophila* obtained from several localities in Hokkaido were investigated in both male and female germ cells with the sectioned material of gonads. The male chromosome patterns of the studied species are given in Table 1.

Table 1. Male chromosome patterns of *Drosophila* studied

Species	2*n*	*n*	Formula
Hirtodrosophila			
D. alboralis	12	6	8R's+2D's+R(X)+R(Y)
Dorsilopha			
D. busckii	6	3	4V's+R(X)+R(Y)
Sophophora melanogaster group			
D. rufa	8	4	4V's+2D's+R(X)+R(Y)
Drosophila quinaria group			
D. nigromaculata	12	6	8R's+2D's+R(X)+R(Y)
virilis group			
D. virilis	12	6	8R's+2D's+R(X)+R(Y)
robusta group			
D. sordidula	10	5	4R's+2v's+2D's+V(X)+V(Y)
Drosophila sp.	12	6	8v's+2D's+V(X)+V(Y)

V : median or submedian chromosome of large size. v. : small median or submedian chromosome. R : terminal chromosome. X : X-chromosome. Y ; Y-chromosome.


x x 1
y x 2
xy 3
4
x x 5
y x 6
xy 7
xy 8
y 9
x 10
x x 11
x y 12
xy 13
14
x x 15
x y 16
xy 17
xy 18
19
20
21
22
xy 23
24
25
x x 26
27
xy 28
xy 29
30
x x 31
32
xy 33
34

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Explanation of Text-figures 1 to 34

Metaphase chromosomes of germ cells in *Drosophila* excepting Fig. 24 which shows early anaphase chromosomes. All figures are camera lucida drawings. Magnification : ca. 4500 ×.

Figs. 1–4. *D. alboralis*. 1, Oogonium. 2, Spermatogonium. 3. Primary spermatocyte (polar view). 4, Primary spermatocyte (side view). Figs. 5–10. *D. buschii*. 5, Oogonium. 6, Spermatogonium. 7, Primary spermatocyte (polar view). 8, Primary spermatocyte (side view). 9, Secondary spermatocyte (Y-class). 10, Secondary spermatocyte (X-class). Figs. 11–14. *D. rufa*. 11, Oogonium. 12, Spermatogonium. 13, Primary spermatocyte (polar view). 14, Secondary spermatocyte. Figs. 15–20. *D. nigromaculata*. 15, Oogonium. 16, Spermatogonium. 17, Primary spermatocyte (polar view). 18, Primary spermatocyte (side view). 19–20, Secondary spermatocyte (X- and Y-class). Figs. 21–25. *D. virilis*. 21, Oogonium. 22, Spermatogonium. 23, Primary spermatocyte (polar view). 24, Primary spermatocyte (side view). 25, Secondary spermatocyte. Figs. 26–30. *D. sordidula*. 26, Oogonium. 27. Spermatogonium. 28, Primary spermatoctye (polar view). 29, Primary spermatocyte (side view). 30, Secondary spermatoctye. Figs. 31–34. *D.* sp. 31, Oogonium. 32, Spermatogonium. 33, Primary spermatocyte. 34, Secondary spermatocyte.