DROSOPHILA SURVEY OF HOKKAIDO. XXIII. A KARYOTYPE STUDY IN SEVEN SPECIES OF THE *QUINARIA* AND THE *ROBUSTA* GROUPS^{1),2)}

TAKASHI TOKUMITSU, TOSHIO SHIMA*, AND AKASI KANEKO

Zoological Institute, Hokkaido University, Sapporo, and *Hokkaido Science Education Center, Sapporo

Received July 3, 1967

A good deal of information on the chromosomes is available to date in the literature on *Drosophila* genetics in relation to species differentiation. It is generally accepted that the differentiation of species has gone hand in hand with the differentiation of the chromosomal mechanism. In recent years chromosome cytology has made surprising contributions to the development of animal systematics particularly in insects. Chromosomal differences are often useful to distinguish cryptic species that cannot be separated morphologically by non-cytogeneticists (White 1954). Indeed, cytogenetical analyses have influenced taxonomy with a new meaning and impetus.

This paper describes preliminarily the results of chromosome observations in seven species belonging to two species groups of the subgenus *Drosophila*: the *quinaria* and the *robusta* species groups. Five species have so far been recorded in Japan in the former group, while a few new members of the latter group have been described at present from Japan (Kaneko *et al.* 1964, Kaneko and Takada 1966).

MATERIALS AND METHODS

Four species of the *quinaria* species group and three species of the *robusta* species group came under study (Table 1). Flies from strains established in our laboratory furnished materials for this study. The chromosome studies were carried out for the most part with oesophageal ganglion cells from the third instar larvae and sometimes with testicular cells of pupae and young adults. Both ganglions and testes were removed in a saline solution under a dissecting microscope. They were fixed and stained with acetic orcein (2%) for 15-30 minutes, and then squashed. Materials treated with a hypotonic solution were observed with phase optics because of their weak affinity to stain.

RESULTS AND REMARKS

1. The *quinaria* species group

1) Contribution No. 778 from Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) This work was made of the partial financial support through a grant to Dr. E. Momma from the Japan Society for the Promotion of Science as a part of the Japan-U.S. Cooperative Science program.

Table 1. Chromosomes of *Drosophila* species under study

Species	2n	Chromosome constitution
<i>quinaria</i> group		
<i>D. unispina</i>	12	10R's + 2D's
<i>D. brachynephros</i>	12	10R's + 2D's
<i>D. angularis</i>	12	10R's + 2D's
<i>D. nigromaculata</i>	12	10R's + 2D's
<i>robusta</i> group		
<i>D. pseudosordidula</i>	10	2V's + 6R's + 2D's
<i>D. sordidula</i>	10	4V's + 4R's + 2D's
<i>D. moriwakii</i>	12	2V's + 2J's + 6R's + 2r's

R: large acrocentric chromosome. D: dot-like chromosome. V: V-shaped (metacentric) chromosome. J: J-shaped (submetacentric) chromosome. r: small acrocentric chromosome.

Figs. 1-7. Chromosomes from male oesophageal ganglion cells. 1; *D. unispina* 2; *D. brachynephros* 3; *D. angularis* 4; *D. nigromaculata* 5; *D. pseudosordidula* 6; *D. sordidula* 7; *D. moriwakii*.

The following four species, *D. nigromaculata*, *D. unispina*, *D. brachynephros* and *D. angularis*, were subjected to chromosomal study. The chromosomes of the first species (*D. nigromaculata*) were reported by Momma (1954). The remaining three species which are closely allied to each other were treated as a single species until Okada's monograph (1956) had been published.

D. unispina: Oesophageal ganglion cells derived from Sapporo-strain specimens showed six pairs of chromosomes as $2n, 12$. The chromosomes except dot-shaped ones exhibited secondary constrictions. A pair of the longest chromosomes, nearly two times as long as the others, showed two distinct secondary constrictions, sometimes bent at the constrictions. This configuration gave the impression as metacentric or submetacentric chromosomes. Four pairs of the rod-shaped chromosomes looked like metacentric elements on account of their constricted features, but anaphasic observations showed them as being of acrocentric nature (Fig. 1).

D. brachynephros: Sapporo-strain of this species had 12 chromosomes in diploid (Fig. 2). The longest chromosomes bear one sometimes two distinct secondary constrictions in each. Other elements of acrocentric nature showed also secondary constrictions, though not specially distinct.

D. angularis: The chromosome configuration of this species from Aomori strain was similar in general appearance to those of *D. unispina* and *D. brachynephros*. The complement comprised six pairs of chromosomes ($2n, 12$) consisting of a pair of dot-shaped elements and five pairs of acrocentric ones (Fig. 3). They did not show distinct secondary constrictions.

It was shown that the above three forms were identical with each other in their karyotypes, except in relative length and in the nature of the secondary constriction. *D. unispina* showed that the chromosomes had clear constrictions, and that the members of the longest pair were as twice in length as the members of four other pairs, while in *D. brachynephros*, the length of the longest chromosomes was about 1.5 times the members of the other acrocentric pairs. The chromosomes of *D. angularis* did not show distinct secondary constriction. The difference in length of the acrocentric elements was not so remarkable in this species as in the above two species. It was noted that in these three species, four pairs of an acrocentric chromosomes except the longest ones were generally indistinguishable from each other, being apparently similar in length.

D. nigromaculata: Flies from Aomori-strain showed twelve chromosomes in diploid which consisted of a pair of dot-shaped elements and five pairs of acrocentric ones of varying lengths. Particularly noticeable is that sex chromosomes were easily identified in this species, a long acrocentric X with a submedian constriction and a Y chromosome which is a long acrocentric one carrying a satellite (Fig. 4). The chromosome configuration of this species was the same as that reported by Momma (1954).

2. The *robusta* species group

The karyotypes of *D. sordidula* (Kikkawa and Peng 1938, Momma 1954) and *D. lacertosa* (Momma 1954) were described previously in this group.

D. pseudosordidula: This species was first collected in Nopporo in 1962 and reported as *D. sordidula* (Kaneko and Tokumitsu 1963). Further detailed examinations

designated this species as a new species by Kaneko *et al.* (1964).

Both oesophageal ganglion cells and spermatogonial cells showed ten chromosomes as diploid which consisted of a pair of metacentric elements, three pairs of acrocentrics and a pair of dot-shaped ones (Fig. 5). Some acrocentric chromosomes observed in the ganglion cells were bent at the part of the secondary constriction. Further, some acrocentrics observed in spermatogonial cells were separated at the centromere, appearing as two separated entities.

D. sordidula: For comparison with *D. pseudosordidula*, the chromosomes of this species were studied in progenies from a female collected in Sapporo. The chromosomes, ten in number, consisted of a pair of metacentric elements, a pair of submetacentric ones, two pairs of acrocentrics and a pair of dot-shaped ones (Fig. 6). The same feature was reported by Kikkawa and Peng (1938) and Momma (1954). One pair of the acrocentrics was characterized by a secondary constriction locating at the region about two-fifth from the distal end. It is thus evident that karyotypically the present species is distinguishable from the former.

D. moriwakii: The chromosomes of this species were observed in male ganglion cells of a strain established from a female collected in Nopporo. The diploid complement showed 12 chromosomes consisting of a pair of metacentric elements, a pair of submetacentric ones, three pairs of acrocentric and a pair of small acrocentrics (Fig. 7). No dot-like element was observed.

SUMMARY

A karyotype study was done in four species of the *quinaria* group, and three species of the *robusta* group of *Drosophila*, with squash preparations of oesophageal ganglion cells and in some cases in spermatogonial cells. The results are summarized in Table 1.

ACKNOWLEDGEMENTS

The authors wish to express their cordial thanks to Professor Sajiro Makino and Assistant Professor Eizi Momma for their valuable suggestions in carrying out the present study and for going through the manuscript.

LITERATURE CITED

- Kaneko, A., and T. Tokumitsu, 1963 Seasonal fluctuations of *Drosophila* populations in two adjacent localities of Hokkaido. *Zool. Mag.* **72**: 123-127.
- Kaneko, A., T. Tokumitsu, and H. Takada, 1964 *Drosophila* survey of Hokkaido, XX. Description of a new species, *Drosophila pseudosordidula* sp. nov. (Diptera, Drosophilidae). *J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* **15**: 374-377.
- Kaneko, A., and H. Takada, 1966 *Drosophila* survey of Hokkaido sp. nov. (Diptera, Drosophilidae). *Annotationes Zool. Japon.* **39**: 55-59.
- Kikkawa, H., and F. T. Peng, 1938 *Drosophila* species of Japan and adjacent localities. *Japan. J. Zool.* **7**: 507-552.
- Momma, E., 1954 *Drosophila* survey of Hokkaido, II. Chromosomes of seven species. *J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* **12**: 200-208.
- Okada, T., 1956 Systemtic study of Drosophilidae and allied families of Japan. Gihodo Co., Tokyo.
- White, M. J. D., 1954 Animal cytology and evolution. The Cambridge University Press.