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A Karyotype Study on the *Drosophila robusta* Species-Group (Diptera: Drosophilidae)

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ABSTRACT—The karyotypes of the *Drosophila robusta* species-group, especially its species-subgroup *okadai*, were studied by using brain cell-Giemsa staining method. *Drosophila gani* possessed a diploid number of 12 chromosomes, comprising 4 pairs of metacentric and 2 pairs of acrocentric chromosomes, and both *D. okadai* and *D. neokadai* did 2n =12, with 3 pairs of metacentrics, 2 pairs of acrocentrics and 1 pair of microchromosomes. The *okadai* subgroup characteristically retained a middle-sized acrocentric X chromosome, which implies that this subgroup may occupy an ancestral position for the *robusta* group.

INTRODUCTION

The *Drosophila robusta* species-group which is widely distributed in the Palearctic and the Nearctic regions has been well studied from micro- and macro-evolutionary points of view (for a review see, Levitan, 1982). Narayanan (1973) has proposed a hypothetical phylogenic tree of the *robusta* group, based mainly on interspecific comparisons of karyotypes and of banded sequences of salivary gland chromosomes. However, not any species of its species-subgroup *okadai* has been studied chromosomally, because of its stubbornness in the laboratory rearing. Further, new members of the *robusta* group have recently been discovered in the mainland of China and new knowledge on its geographic distribution has been accumulated in China and Russia (Watabe *et al.*, 1990a; Watabe and Peng, 1991; Chen and Watabe, 1993; Toda *et al.*, 1996).

In the present article, first we investigated the results of the chromosomal observations of the *robusta* group, and then discussed an ancestor form common to the *robusta* group and its sister species-group *melanica*, based on the results obtained and recent information on the biogeography of those groups.

MATERIALS AND METHODS

All flies were collected by banana traps and from timber piles from 1994 to 1996. The species and geographic strains available for the present study are given in Table 1, together with the results of the karyological observations and information of the geographic distribution.

The females were reared in glass vials with malt-yeast medium at 18°C, and a small piece of apple was placed on the surface of food for the oviposition site.

Preparations of mitotic chromosomes were made with neuroblasts of the 3rd larvae treated with 0.1 mg/ml of colchicine solution, stained with 4% Giemsa solution, and then air-dried (Imai *et al.*, 1977). About 100 nuclear plates were observed in each species.

RESULTS AND DISCUSSION

Figure 1 shows the metaphase configurations of five species of the *robusta* species-group and *D. moriwakii* of the *melanica* group. The karyotype of *D. okadai* was composed of 3 pairs of middle-sized metacentrics (V-shaped), 2 pairs of acrocentrics (Rod-shaped), and 1 pair of dotlike (D) chromosomes (Fig. 1A). Its X chromosome was acrocentric and the Y was metacentric. *Drosophila neokadai* showed the same karyotype as *D. okadai*, except for its submetacentric (J-shaped) Y chromosome (Fig. 1B). *Drosophila gani* possessed 2n =12 chromosomes, consisting of 4 pairs of middle-sized metacentric autosomes, 1 pair of acrocentric autosomes, and unpaired sex chromosomes, acrocentric X and metacentric Y (Fig. 1C). These three species belong to the *okadai* subgroup, together with *D. unimaculata* distributed in eastern and central Europe (Watabe and Nakata, 1989). The metaphase plates of *D. sordidula* of the *robusta* subgroup comprised 2 pairs of metacentrics, 2 pairs of acrocentrics and 1 pair of dotlike chromosomes (Fig. 1E). The same result has been already obtained by Kikkawa and Peng (1938). The X chromosome of *D. sordidula* is the largest metacentric, being about two times as long as acrocentric autosomes. Such a large metacentric X has been found in three species of the *robusta* subgroup, *D. robusta, D. pseudosordidula* and *D. cheda* (Tan *et al.*, 1949; Tokumitsu *et al.*, 1967), and in one species of the *lacertosa* subgroup, *D. lacertosa* with some variations of small auto-

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Table 1. Karyotypes of the *Drosophila robusta* and the *D. melanica* species-groups, and their geographic distribution

Species-group	Metaphase chromosome[1]			Collection[2]	Distribution
Species	2n	karyotype	sex chromosome	localities	
the *robusta* group					
D. okadai Takada	12	3V, 2R, 1D	X=R Y=V	JZ, SR	Japan, RFE (Russian Far East)
D. neokadai Kaneko & Takada	12	3V, 2R, 1D	X=R Y=J	JZ, SR	Japan, ne. to sw. China, RFE
D. gani Liang & Zhang	12	4V, 2R	X=R Y=V	MM, OG, KG	Japan, sw. China
D. sordidula Kikkawa & Peng	10	2V, 2R, 1D	X=V Y=V	US, MS	Japan, Korea, ne. China, RFE
D. lacertosa Okada	10	5V	X=V Y=V	TN, KK, TK NG, US, CH	Japan, Korea, ne. to sw. China Taiwan, Myanmar, Nepal, India
the *melanica* group					
D. moriwakii Okada & Kurokawa	12	2V, 4R	X=R Y=V	JZ	Japan, ne. China, RFE
D. tsigana Burla & Gloor	10	2V, 2R, 1D	X=R Y=J	MS	Japan, Korea, ne. and sw. China, RFE, w. Europe (Pyrénée Mountains)

[1] Abbreviations of chromosomes are explained in the text.

[2] JZ (Jyozankei), SR (Mt. Soranuma), MS (Misumai),TN (Tonebetsu) and KK (Kikonai) in Hokkaido; MM (Mimmaya) in Aomori Pref.; TK (Mt. Takao) in Tokyo; KG (Kanagi) in Shimane Pref.; NG (Nogouchi) in Fukuoka Pref.; JS (Jusou) in Kagoshima Pref.; US (Ussuri Nature Reserve) in RFE; CH (Chitou) in Taiwan.

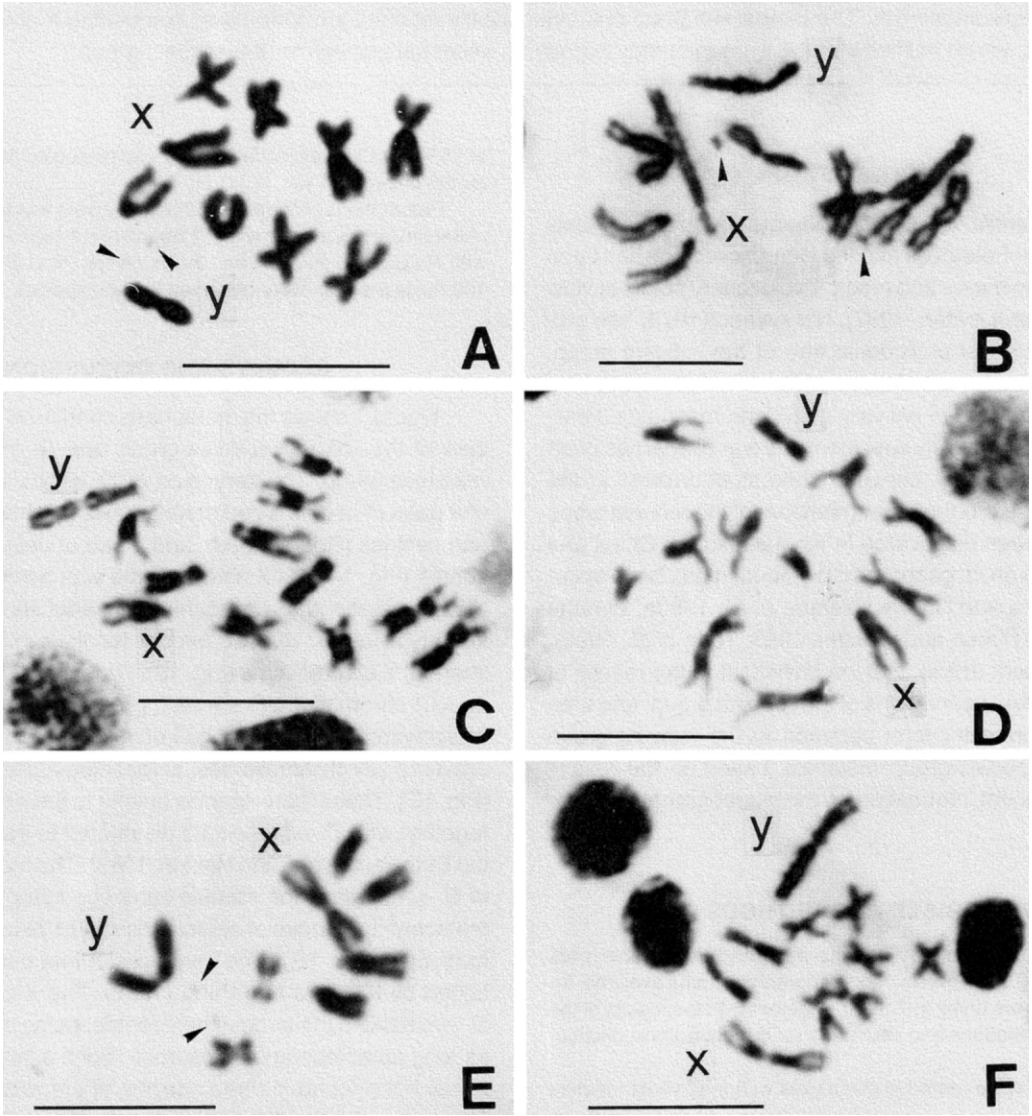

Fig. 1. Male metaphase chromosomes of the *Drosophila robusta* species-group and *D. moriwakii*. **A**, *D. okadai*; **B**, *D. neokadai*; **C**, *D. gani*; **D**, *D. moriwakii*; **E**, *D. sordidula*; **F**, *D. lacertosa*. Arrowheads indicate microchromosomes. Bars=10 µm.

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somes (cf. Momma, 1956; Fig. 1F).

In addition to the *robusta* group, the karyotypes of two species of the *melanica* group, *D. moriwakii* and *D. tsigana*, were checked for the following discussion. *Drosophila moriwakii* shows a slight variation of the chromosome complement, i.e., 2n =12 with 3V + 2R + 1D (Okada and Kurokawa, 1957), 2n =12 with 1V + 1J + 4R (Tokumitsu *et al.*, 1967), 2n =12 with 2V + 4R (Fig. 1D) and 2n =14 with 2V + 4R +1D (Watabe, unpubl.), but its X has been observed invariably to be acrocentric in those articles. The karyotype of *D. tsigana* comprised 2n =10 (2V + 2R +1D) with middle-sized acrocentric X, and the same result was reported as in "*melanissima*" by Kikkawa and Peng (1938) and as in "*pengi*" by Okada and Kurokawa (1957).

The rod-shaped X has been considered to be primitive than the large V-shaped one, since centric fusion with the loss of one centromere is much more likely to occur in the speciation of *Drosophila* than centric fission with the addition of one centromere. Geographical, historical, or genetical evidences support this in various groups of *Drosophila*, e.g. in Hawaiian *Drosophila* (Carson and Yoon, 1982). With regards to the *robusta* group, all autosomes are middle-sized, whereas large metacentric X are found in the both *robusta* and the *lacertosa* subgroups. The single-element rod X had been known only in two allopatric species, Asiatic *D. moriwakii* and North American *D. colorata*, and therefore Narayanan (1973) considered that they "are, or represent" the ancestor species of the *robusta* group. Beppu (1988) transferred *D. moriwakii* and *D. colorata* from the *robusta* group to its closely related species-group *melanica*, based on the genitalial structure, although the two species share all the diagnostic characters of the *robusta* group defined by Sturtevant (1942). If this is true, a primitive form of the *robusta* group will become open to question, again. The present results, i.e., the presence of acrocentric X in the *okadai* subgroup and a large V-shaped one in the other subgroups, strongly suggest that the former may occupy an ancestral position in the *robusta* group (Fig. 2). In addition to the morphology of X, the chromosome complement of the *okadai* subgroup resembles that of *D. moriwakii* and *D. colorata*, in having the same number of chromosomes (2n =12) and in lacking any of large-sized metacentric chromosomes (Fig. 1A–D). Hence, it is suggested by the outgroup comparison that the ancestor common to both the *robusta* and the *melanica* groups may have possessed a rod-shaped X, and subsequently Robertsonian translocation and pericentric inversions may have occurred during the course of species divergence within the *robusta* group and the *melanica* group.

The rod-shaped X is also known in other species of the *melanica* group, Palearctic *D. tsigana* and North American *D. micromelanica* (Stalker, 1972). *Drosophila tsigana* presently shows a disjunctive pattern of the geographic distribution, East Asia and western Europe (Watabe *et al.*, 1990b). It is highly probable that *D. tsigana* might have once possessed an extremely wide range of distribution and that its American relatives might have diversified from "pre-*tsigana*".

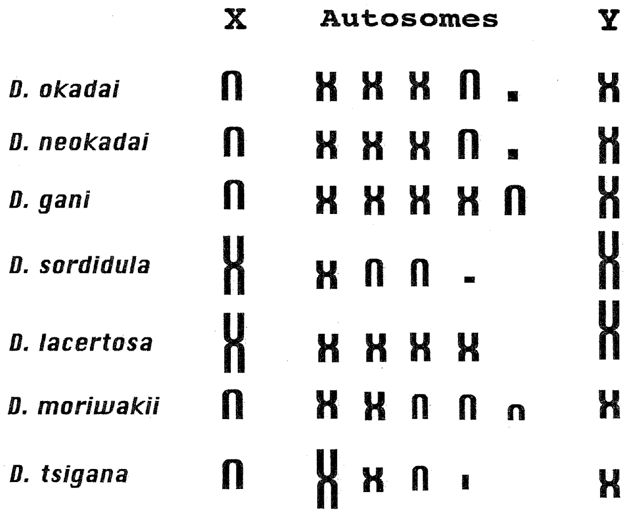

Fig. 2. Schematic representation of chromosomes in the *Drosophila robusta* group and the *D. melanica* group.

A remarkable decrease of temperature called "Terminal Eocene Event" took place from late Eocene to early Miocene (ca. 38.6–16.3 mya), and this reinforced the range expansion of temperate mixture forests in the middle latitudes of Eurasia (Tanai, 1971,1990; Wolfe, 1978). The *robusta* and the *melanica* species-groups might have emerged in East Asia during this Tertiary period (Throckmorton, 1975), since both groups are the temperate species in the Holarctic region and are strongly sylvicolous. Thereafter, temperate forests spread to high latitudes of Eurasia in early to mid Miocene (ca. 16.3–10.4 mya) when the warming of temperature occurred, and the *robusta* and the *melanica* group flies might have immigrated from Eurasia to North America, via the Bering Straight.

The recent faunal studies in the mainland of China and in the Russian Far East have yielded many new species and a lot of knowledge available on the biogeography of the *robusta* group and its related species-groups (Watabe *et al.*, 1990a; Watabe and Peng, 1991; Chen and Watabe, 1993; Toda *et al.*, 1996). A further investigation using molecular techniques is obviously desirable for tracing accurately the phylogeny and evolution within and between the *robusta* and the *melanica* species-groups.

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