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Author(s)	渡部，英昭
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A Karyotype Study on the *Drosophila virilis* section (Diptera, Drosophilidae)

WATABE Hideaki

Biological Laboratory, Sapporo Campus, Hokkaido University of Education, Sapporo 002-8502

クロショウジョウバエ区の核型研究

渡　部　英　昭

北海道教育大学札幌校生物学教室

ABSTRACT

Chromosomal compositions were studied in six members belonging to the *Drosophila* (*Siphlodora*) *virilis* section. In the *robusta* species group, *Drosophila yunnanensis* had 2n=10 chromosomes with 3 pairs of metacentric, 1 pair of submetacentric and 1 pair of acrocentric chromosomes. Its sibling *D. medioconstricta* had 2n=12 chromosomes, comprising 2 pairs of metacentric, 2 pairs of submetacentric, 1 pair of acrocentric, and 1 pair of micro-chromosomes. X chromosomes were metacentric in both species. Two species of the *polychaeta* species group, *Drosophila daruma* and *D. latifshahi*, possessed the same karyotype of 2n=12, 3 pairs of metacentric, 2 pairs of acrocentric and 1 pair of micro-chromosomes. X chromosomes were acrocentric in those species. Ganglion cells of *Drosophila angor* showed 2n=12, 2 pairs of metacentric, 3 pairs of acrocentric and 1 pair of micro-chromosomes (X=Rod-shaped), and those of *Drosophila fluvialis*, an Asian member of the *Drosophila virilis* section showed 2n=12, 4 pairs of metacentric and 2 pairs of acrocentric chromosomes (X=R), lacking micro-chromosomes. This is the first record on the karyotype of the *angor* and the *fluvialis* group in the *Drosophila virilis* section.

Key words: Chromosomes, *Drosophila*, *angor* group, *Drosophila fluvialis*, East Asia

1. INTRODUCTION

Chromosomal analyses provide us with invaluable information, when we consider the phylogenetic relationships of drosophilid flies (Patterson and Wheeler, 1942; Clayton and Wheeler, 1975; Clayton and Guest, 1986; Throckmorton, 1982; He et al., 2003; Wang et al.,

2006a, b). Since the 1940's, chromosomes of the *Drosophila virilis* section flies, e.g., the *virilis*, the *robusta* and the *melanica* species groups, have been intensively studied from a karyotypic evolutionary point of view, mainly by North American researchers (Patterson et al., 1940; Sturtevant, 1942; Stalker, 1942, 1966; Narayanan, 1973; Levitan, 1982, and *etc.*). The *Drosophila virilis* section established by Hsu (1949) or the *virilis-repleta* Radiation by Throckmorton (1975) is one of the major lineages in the evolution of the genus *Drosophila*, and Yassin (2013) has transferred this taxonomic group from the subgenus *Drosophila* to the subgenus *Siphlodora* in the genus *Drosophila*.

A number of new members of the *Drosophila virilis* section have been discovered from southern China and Southeast Asia from the end of the 20th century, and the new species groups are established in this taxon (Toda and Peng, 1989; Watabe et al., 1990a, b; Watabe and Peng, 1991, Suwito and Watabe, 2010; Suwito et al., 2014). Most of the *virilis* section flies inhabit highlands with cool climates in low latitudes, and it is difficult to establish living strains of the *virilis* section, because most of the wild-collected females are dead during transportation from fields to laboratories. Further, the rearing method for the *angor* group has not yet been established, which is an Asiatic member of the *Drosophila virilis* section distributed in the lowlands of tropical and subtropical zones.

The author has had a chance to examine chromosomes of some of the *Drosophila virilis* section flies, and the results on the karyotype analysis are shown in the present article.

2. MATERALS AND METHODS

All of the adult flies were collected by fermenting banana traps set along streams in southern Japan and Taiwan. The collection localities are given in Table 1, together with the results of karyotypic observations. Wild-caught females were slightly etherized for identifying species, and transferred to the glass vial with the usual medium for the *Drosophila* culture, comprising yeast, cornmeal, sucrose, malt and agar. A small piece of apple was placed on the surface of the medium for female's oviposition. Larvae were maintained in incubators at 20℃. Neuroblasts of 3rd instar larvae were treated with 0.1 mg/ml of colchicine solution for 30 min, fixed with a solution (carbinol: acetic acid = 3:1) for 1 hour, stained with 4% Giemsa solution for 40 min, and then air-dried (Imai et al., 1977). About 100 nuclear plates were examined in two members of the *polychaeta* species group, and about 30 nuclear plates were studied in the other groups, owing to difficulties of laboratory rearing. Metaphase chromosomes were taken by an analog camera (Olympus PM-6) and then saved as digital figures using a film-scanner (Nikon APS IX240).

3. RESULTS AND DISCUSSION

Figure 1 shows chromosomal configurations of six species of the *Drosophila virilis* section. No chromosomal variation was observed among geographic populations of those species used in this study. Male metaphase plates of *Drosophila yunnanensis* Watabe, Liang and Zhang, 1990 showed 2n=10, consisting of 3 pairs of metacentric chromosomes, 1 pair of middle-sized submetacentric autosomes (J-shaped) and 1 pair of acrocentric autosomes (Rod-shaped). Its X chromosome was large metacentric (V-shaped), with large areas of heterochromatin around the centromere. Y was large metacentric

(Fig. 1A). The karyotype of *Drosophila medioconstricta* Watabe, Liang and Zhang, 1990 was composed of 2n=12, 2 pairs of metacentric, 2 pairs of submetacentric, 2 pairs of acrocentric chromosomes and 1 pair of micro-chromosomes (Dot-like). Both X and Y chromosomes were metacentric (Fig. 1B). Both *Drosophila yunnanensis* and *D. medioconstricta* belong to the *lacertosa* subgroup of the *robusta* species group. The karyotype of the former species resembles that of the southern China population of *D. lacertosa*, in having 2n=10 and in lacking dot-like chromosomes (He et al., 2003), suggesting a close relationship between these siblings. The karyotype of *D. medioconstricta* is characteristic in the *robusta* group, in having 2 pairs of V-shaped and 2 pairs of J-shaped chromosomes, and no one species is closely related to *D. medioconstricta* in the karyotype. The *robusta* species group is classified into 3 subgroups, the *lacertosa*, the *okadai* and the *robusta* subgroups. It is considered that the rod-shaped X is more primitive than the V-shaped one in the chromosomal evolution of the *robusta* group, and most members of the *okadai* subgroup possess the primitive type of acrocentric X chromosome (Watabe et al., 1997). Many new members of the *robusta* group have been discovered in mainland China and Southeast Asia (Watabe et al., 1990a; Suwito and Watabe, 2010), and a further analysis is needed to trace the karyotype evolution in the *robusta* group.

Figures 1C and D show the male metaphase plates of *Drosophila daruma* Okada, 1956 and *D. latifshahi* Gupta and Ray-Chaudhuri 1970, respectively. *Drosophila latifshahi* retained 2n=12, with 3V+2R+1D (X=R). Chromosomes of this species have already been reported by Gupta (1973) and by Singh and Gupta (1979) under the species name of "*Scaptodrosophila latifshahi*", and later Toda and Peng (1989) transferred this species from the genus *Scaptodrosophila* to the genus *Drosophila*. The present result is identical to that of Gupta (1973). Larval ganglion cells of *Drosophila daruma* also showed 2n=12 (3V+ 2R+1D; X=R). The karyotype of *D. polychaeta* itself was reported to be 2n=12, 2R+2J+1V+1D by Patterson and Wheeler (1942). Metacentric chromosomes might have been frequently regarded as submetacentric in different directions of observation, and thus it may be said that all members of the *polychaeta* group species have the same karyotype with acrocentric X chromosome.

The *Drosophila angor* group was established by Watabe and Peng (1989), with the name of the *wakahamai* group. Later, Chen and Watabe (1993) examined the type specimen of *D. angor* Lin and Ting, 1971, and concluded that "*Drosophila wakahamai* Toda and Peng, 1989" was a junior synonym of *D. angor* in the subgenus *Drosophila*. It was very difficult to maintain *D. angor* under laboratory conditions, and thus quite a limiting number of 3rd larvae were obtained. The ganglion cells of *D. angor* retained 2n=12, 2V+3R+1D. Its X chromosome was acrocentric. This is a first record on the karyotype of the *angor* species group.

Drosophila fluvialis Toda and Peng, 1989 is an Asian member of the *virilis* section, and its habitat is restricted to riparian environments with high humidity in subtropical and tropical regions. Most adult females did not deposit their eggs on the medium in the glass vial, and it was nearly impossible to establish living strains of *D. fluvialis*. The limited number of 3rd larvae, about 20 each for sex, were examined chromosomally in the present study. The male metaphase

plates showed 2 pairs of large sized metacentric, 2 pairs of middle-sized metacentric and 2 pairs of acrocentric chromosomes (Fig. 1F). The X chromosome was acrocentric. *Drosophila fluvialis* lacks micro-chromosomes, common to many drosophilid flies.

As mentioned previously, many new members of the *Drosophila virilis* section have been discovered in southwestern parts of mainland China, especially from the Yun-Gui Highlands, and in southeastern Asia including Indonesia, Malaysia and Philippines. The *angor* species group is very abundant in riparian environments of the lower latitudes of Asia and some species closely related to *D. fluvialis* are known from tropical regions. A further study on the karyotype is necessary for clarifying the evolutionary process of those species.

Table 1. Collection data of the *Drosophila virilis* section flies used for the present study.

Species group	Species name	Collection data		Chromosome constitution*
		locality	date	
robusta	*D. yunnanensis*	Fushan, Taiwan, China	19.iv.1997	2n=10 (3V, 1J, 1R), X=V, Y=V
		Chitou, Taiwan, China	22.iv.1997	
	D. medioconstricta	Fushan, Taiwan, China	19.iv.1997	2n=12 (2V, 2J, 1R, 1D), X=V, Y=V
polychaeta	*D. daruma*	Yona, Kunisaki, Okinawa, Japan	26.iii.1996	2n=12 (3V, 2R, 1D), X=R, Y=V
		Mt. Omoto, Ishigaki, Japan	22.iii.2016	
	D. latifshahi	Nankang, Taiwan, China	18.iv.1997	2n=12 (3V, 2R, 1D), X=R, Y=V
		Konti, Taiwan, China	22.iii.1997	
angor	*D. angor*	Yona, Kunisaki, Okinwa, Japan	28.iii.1996	2n=12 (2V, 3R, 1D), X=R, Y=R
		Mt. Omoto, Ishigaki, Japan	10.iii.2004, 22.iii.2016	
		Chitou, Taiwan, China	22.iv.1997	
ungrouped	*D. fluvialis*	Mt. Omoto, Ishigaki, Japan	23.iii.1996	2n=12 (4V, 2R), X=R, Y=J
		Mahre R., Iriomote, Okinawa, Japan	18.iii.2008	

*See the text for abbreviations of chromosome types.

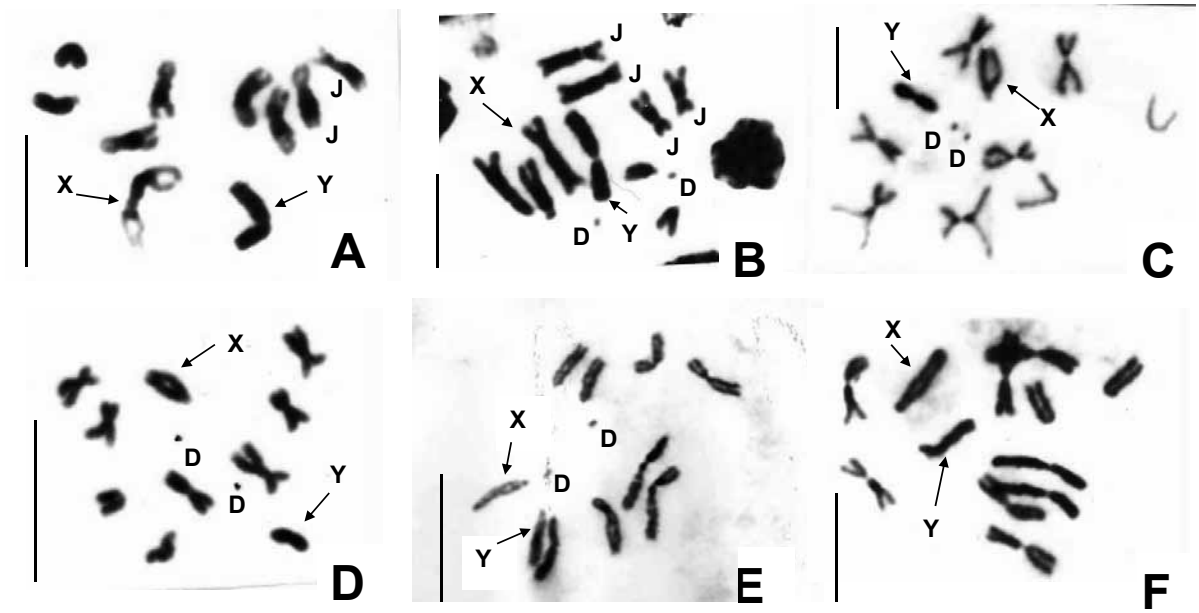

Figure 1. Chromosome configurations of six species of the *Drosophila virilis* section. A: *Drosophila yunnanensis* from Chitou, B: *D. medioconstricta* from Fushan, C: *D. daruma* from Iriomote Is., D: *D. latifshahi* from Nankang, E: *D. angor* from Yona, F: *D. fluvialis* from Mt. Omoto, Ishigaki. Bars=10μm.

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（札幌校教授）