

Sexual isolation studies of *Drosophila kanekoi*

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ABSTRACT

Interspecific crossability between *Drosophila kanekoi* and other members of the *D. virilis* group was studied by using no choice and female choice methods. Asymmetric mating preference was observed in every cross. Females of *D. kanekoi* more frequently mated to males of *D. virilis*, *D. lummei* and of *D. montana montana* than in the reciprocal crosses. A complete sexual isolation was found between two sympatric species, *D. kanekoi* and *D. ezoana*.

1. INTRODUCTION

The *virilis* species group consists of eleven species, some of which include subspecies. They are distributed in both Palaearctic and Nearctic regions, except for a domestic, world-wide species of *D. virilis*. Evolution of this group has extensively been studied by means of sexual isolation (Stalker 1942b), interspecific chromosome analysis, morphological and biochemical comparisons (Stone *et al.* 1960; Throckmorton 1982). *Drosophila kanekoi* has recently been described from Hokkaido, Japan (Watabe and Higuchi 1979). It is often observed in Hokkaido that *D. kanekoi* and *D. ezoana* are sympatrically distributed particularly in riparian zone. This paper presents the crossability between *D. kanekoi* and the other members of this species group.

2. MATERIALS AND METHODS

Seven iso-female lines were used. These were as follows; *D. virilis* (Sapporo, Japan), *D. lummei* (Oulu, Finland), *D. littoralis* (Strängnäs, Sweden), *D. ezoana* (Sapporo, Japan), *D. montana montana* (Cottonwood Canyon, Utah, U. S. A.), *D. montana ovivororum* (Kiuruvesi, Finland) and *D. kanekoi* (Chitose, Japan).

No Choice Method: Newly emerged flies were etherized every day and sorted for sex, and were stored in vials (40mm in diameter, 120mm in height) with usual yeast medium at room temperature (18-30°C) for one to two days.

Table 1. *Frequencies (%) of inseminated females in no choice method and production of hybrids between the members of the Drosophila virilis group. F: hybrids produced (F₁), N: no hybrid produced*

female	male						
	Vi	Lu	Lt	Ez	Mo	Ov	Ka
<i>D. virilis</i> (Vi)	—	58.6 (31) F	90.3 (31) F	3.0 (33) N	47.3 (39) F	34.8 (23) F	26.2 (80) N
<i>D. lummei</i> (Lu)	88.8 (27) F	—	62.5 (32) F	0.0 (26) N	72.7 (55) F	32.2 (31) N	17.0 (47) N
<i>D. littoralis</i> (Lt)	82.7 (29) N	11.7 (34) N	—	20.0 (55) N	40.0 (40) F		12.0 (75) N
<i>D. ezoana</i> (Ez)	4.1 (24) N	0.0 (22) N	35.5 (34) N	—	51.0 (35) N	35.8 (39) N	0.0 (27) N
<i>D. montana montana</i> (Mo)	50.0 (38) F	33.3 (27) N	3.2 (39) F	20.5 (34) N	—		0.0 (35) N
<i>D. montana ovivororum</i> (Ov)	8.0 (23) N	23.3 (30) N		0.0 (29) N		—	0.0 (20) N
<i>D. kanekoi</i> (Ka)	84.5 (58) N	42.8 (56) F	77.7 (18) N	1.1 (26) N	86.6 (60) F	0.0 (31) N	—

The numbers in parentheses give the number of females examined.

Then, five males and the same number of alien females were placed together in a vial (30 mm in diameter, 120 mm in height) and reared for 30 days at 18°C under continuous light. Thereafter, females were dissected and examined for sperm in both spermathecae and seminal receptacles. Six to twelve replicates were made for each cross. In addition, the culture vials were also examined for presence of larvae.

Female Choice Method: Five males were placed together with ten females, five of conspecific females and five of alien ones, in a vial. They were reared for one day at 18°C under continuous illumination. There were clear differences from species to species in the period of sexual maturation; *D. virilis* and *D. lummei* females began to mate before the maturation of the ovaries, while *D. kanekoi* and *D. m. montana* females did not mate before their ovaries were fully developed. Therefore, 24-days old flies were used for every cross. The degree of sexual isolation was indicated by the isolation index (I.I.) (Stalker 1942b).

3. RESULTS AND DISCUSSION

Table 1 shows the results of the crossability by using no choice method. The females of *D. kanekoi* mated well to the males of *D. virilis*, *D. lummei*, *D. littoralis* and of *D. m. montana*, but scarcely to those of *D. ezoana* and *D. m. ovivororum*. On the other hand, the males of *D. kanekoi* hardly mated to

Table 2. Sexual isolation between *Drosophila kanekoi* (Ka) and three species, *D. virilis* (Vi), *D. lummei* (Lu) and *D. montana montana* (Mo) of the *virilis* group

Cross		Homogamic		Heterogamic		% of Homo-	% of Hetero-	I. I.	
Female	Male	(+)	(-)	(+)	(-)	gamic (+)	gamic (+)		
Ka	Vi	Ka	60	0	0	60	100	0	1.00
Ka	Vi	Vi	47	4	5	54	92.1	10.2	0.80
Ka	Lu	Ka	24	1	8	23	96	34.7	0.46
Ka	Lu	Lu	20	11	2	33	64.5	5.7	0.83
Ka	Mo	Ka	35	0	0	38	100	0	1.00
Ka	Mo	Mo	55	0	23	31	100	42.6	0.40

Homogamic (+) and Heterogamic (+) indicate the number of females inseminated by their own males and by alien males, respectively.

alien females. Both males and females of *D. virilis* well mated to the other species, except for *D. ezoana*. During the cross experiment of *D. kanekoi* females with *D. ezoana* males, 3 *kanekoi* females died by 30th days after their exposure but they had all been inseminated. Since none of alive female had been inseminated in this case, the interspecific insemination seemed to have a lethal role for *D. kanekoi* females. Although *D. kanekoi* and *D. ezoana* are often found to live together particularly in riparian zone, the data represented in this study imply that there is a strong, reproductive isolating mechanism between the species and their gene pools are thus discrete mutually.

Table 2 gives the results of the interspecific sexual isolation by using female choice method. The I. I. values are low between *D. lummei* females and *D. kanekoi* males and between *D. kanekoi* females and *D. m. montana* males. This result is coincident with that obtained in the experiment using no choice method, except for the cross between *D. lummei* females and *D. kanekoi* males.

The *virilis* species group consists of two major lineages, the *virilis* and the *montana* phylads (Throckmorton 1982). Watabe and Higuchi (1979) have reported that *D. kanekoi* has morphologically combined characteristics of the both phylads. In this connection, Throckmorton (personal communication) suggests that *D. kanekoi* has "dot chromosome", a characteristic of the *montana* phylad, while it has a "small ejaculatory bulb", a characteristic of the *virilis* phylad. These facts may reflect the mating preference revealed here that the females of *D. kanekoi* well accepted the males of either of the phylad. Further, by means of protein analysis, Throckmorton (1982) states that this species can be considered to be derived at early stage of the speciation of the *virilis* group. The facts mentioned above lead to conclusion that *D. kanekoi* might be an oldest member which occupies phylogenetically

important position between the two phylads of this group.

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