

## One-sided sexual isolation between *Drosophila takahashii* and *Drosophila pseudotakahashii*

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**Summary.** In order to elucidate the phylogenetic relationships between *D. takahashii* and *D. pseudotakahashii*, 2 closely related allopatric species, sexual isolation was studied by the male-choice method. The present data indicate that there is a one-sided mating preference between these species. On a basis of the results, their evolutionary sequence is discussed.

Sexual isolation and hybrid sterility are the primary mechanisms of reproductive isolation in speciation. These components of speciation are frequently observed among closely related species of *Drosophila*<sup>2</sup>. It is a widely accepted hypothesis that reproductive isolation may arise as a by-product of genetic divergence of incipient species or it may be induced by natural selection as a barrier to gene exchange<sup>3</sup>. Sexual isolation therefore may be expected to occur more often between sympatric than between allopatric species. The mode of sexual isolation has been used to evaluate the direction of evolution among closely related species of *Drosophila*<sup>4,5</sup>.

*D. takahashii* and *D. pseudotakahashii* constitute a pair of allopatric sibling species. *D. takahashii* is widely distributed whereas *D. pseudotakahashii* is endemic to Australia<sup>6,7</sup>. Interspecific hybridization has been reported by Mather<sup>8</sup> who obtained hybrids of both sexes (fertile female and sterile male) in equal number in only one direction when *D. takahashii* was the male parent. However, Dwivedi<sup>9</sup> found hybrids in both directions.

The results of male-choice experiments designed to evaluate the pattern of sexual isolation between the 2 species are reported here.

**Materials and methods.** The isofemale lines of *D. takahashii* and *D. pseudotakahashii* were established in the laboratory from the stock cultures obtained from Japan and Australia respectively. The virgin females and males to be utilized in the experiments were aged for 7 days in small batches. The 'male-choice' method was used. Briefly, this involves the confinement of 1 female of each of the 2 species with 1 male of 1 of these in a food vial. After exposing the females to the male for 5 days, both the females were dissected and their sperm receptacles were examined for the presence or absence of sperm. The isolation index<sup>10</sup> for the 2 species was calculated from the data by taking the difference between homogamic and heterogamic matings and dividing by the total matings.

**Results.** The results of male-choice experiments are report-

ed in the table. The present species show no sexual isolation when a *D. takahashii* male is used (isolation index=0.06). Homogamic and heterogamic matings are nearly equally frequent: the  $\chi^2$  of 1.06 has probability of chance occurrence of more than 0.30 (d.f.=1). The departure from the normal condition of random mating is statistically not significant. In contrast to this, the 2 species show pronounced sexual isolation when a *D. pseudotakahashii* male is used (isolation index=0.62). Heterogamic matings are less frequent than homogamic matings: the  $\chi^2$  of 23.9 has a probability of chance occurrence of less than 0.001 (d.f.=1). The deviation from randomness of mating is highly significant statistically. Thus the results indicate that *D. pseudotakahashii* males are discriminated against by *D. takahashii* females whereas *D. takahashii* males are not discriminated against by *D. pseudotakahashii* females.

**Discussion.** It is evident from the present results that there is one-sided sexual isolation between *D. takahashii* and *D. pseudotakahashii*. The males of *D. pseudotakahashii* are discriminated against by *D. takahashii* females. It is as if *D. pseudotakahashii* males have evolved a specific odour that females of *D. takahashii* find unacceptable, since chemical stimuli often serve as isolating mechanisms<sup>11</sup>. On the other hand, *D. takahashii* males are readily accepted by *D. pseudotakahashii* females and isolation is eliminated.

Two hypotheses have been proposed to interpret the evolutionary sequence on the basis of sexual isolation patterns in closely related species of *Drosophila*. Kaneshiro<sup>4</sup> postulates that females of ancestral species show strong sexual discrimination against males of the more derived species. His hypothesis is based on the founder principle of inter-island speciation<sup>12</sup>. In contrast to this, Watanabe and Kawanishi<sup>5</sup> proposed that it is the females of new species which do not mate with the males of ancestral species. The wide geographical distribution of *D. takahashii*<sup>6,7</sup> and its degree of inversion polymorphism<sup>9,13</sup> indicate that this species is ancestral to *D. pseudotakahashii*. This phylogenetic relationship favours Kaneshiro's hypothesis.

Results of male-choice experiments between *D. takahashii* and *D. pseudotakahashii*

Types of crosses		Homogamic			Heterogamic			I*	$\chi^{2**}$
Females	Male	Tested	Insemi-nated	%	Tested	Insemi-nated	%		
<i>D. takahashii</i> + <i>D. pseudotakahashii</i>	<i>D. takahashii</i>	133	68	51.13	136	61	44.85	0.06	1.06
<i>D. takahashii</i> + <i>D. pseudotakahashii</i>	<i>D. pseudotakahashii</i>	87	37	42.53	89	9	10.11	0.62	23.9

\* Stalker's isolation index. \*\* Calculated from a 2 × 2 contingency table.

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