Mating Preference and the Direction of Evolution in *Drosophila*

Abstract. *Differences in the degree of successful matings between pairs of reciprocal hybrid matings indicate the direction of evolution among related species of* Drosophila. *Females of a derived species do not mate with males of the ancestral species, whereas females of the ancestral species readily mate with males of the derived species.*

Mating preference between species or ethological isolation has been repeatedly observed in *Drosophila*. Females usually choose their mating partners if males of different species are present, while males generally show no mate preference (*1*). There is some question as to whether or not the mode of mating preference can be used to elucidate the direction of evolution. Kaneshiro (*2*) postulated that when a new species arose its males were not readily accepted by females of the original (ancestral) species. In contrast, we propose that it is the females of the new species which do not mate with the males of the ancestral species. Using data collected on mating preference, we present our comparison of these two opposing hypotheses.

To evaluate ethological isolation, sexually mature males and females of different strains were placed in the same vial for a determined period of time. The females were then dissected to determine whether they have been inseminated. The following experiment is termed "male-choice" (*1*). Ten males of strain A were placed with 20 females, ten of strain A, and ten of strain B. In the reciprocal cross, B males were placed with females of A and B. The isolation index (*3*) for the two strains can be calculated from the data by taking the difference between homogamic matings and heterogamic matings and dividing by the total matings. If the index is 1, the two strains are at the level of different species and if the index is 0 or negative, they are at the level of same species.

Two strains of *Drosophila melanogaster* (Oregon-R, Mishima), three strains of *D. simulans* (North Carolina, Tananarive, Mishima) and a strain of *D. mauritiana* (Riviere Noire) were used for the male-choice experiment. *Drosophila mauritiana* is a recently discovered species closely resembling *D. simulans* both genetically and cytologically (*4*). Two-day-old flies were placed together for 2 days (Table 1). Since there was no significant difference among strains of each species, within-species data were pooled. A clear difference in the isolation index was found between reciprocal matings of each pair of species (*5*).

Melanogaster females were always successful in mating with *simulans* and *mauritiana* males than the reciprocals (*simulans* females were crossed with *melanogaster* males, and *mauritiana* females were crossed with *melanogaster* males). In contrast, *simulans* females were more successful in mating with *mauritiana* males than the reciprocal cross of *mauritiana* females with *simulans* males, but were less successful with *melanogaster* males than the reciprocal. Finally, *mauritiana* females were always less successful in mating with both *melanogaster* and *simulans* males than were the reciprocal crosses (Fig. 1A). If we hypothesize that females of a newly evolved species do not mate well with males of the ancestral species, the direction of evolution would seem to be *melanogaster* → *simulans* → *mauritiana*. However, according to Kaneshiro's hypothesis, the evolutionary sequence would be *mauritiana* → *simulans* → *melanogaster*.

The direction of evolution in the *virilis* group of *Drosophila* has been discussed on the basis of cytological (*6*), morphological (*7*), and electrophoretic (*8*) studies. Investigations of these different features have resulted in the conclusion that *virilis* is the ancestral species and that the other species have been derived as follows: *virilis* → *novamexicana* → *americana* → *texana*, and *virilis* → *lacicola* and *montana*. Table 1 also shows the reciprocal isolation indices obtained from two reports (*9*). A marked difference in the index is found between reciprocal crosses of each species pair. For example, females of *virilis* mated well with males of other species while *virilis* males rarely mated with other species females. The difference between reciprocal matings of each pair of species can be represented in the same manner as the *melanogaster* group (Fig. 1A). Figure 1B shows that *virilis* is the ancestral species and that *novamexicana* evolved from it; *americana* and *texana* were subsequently derived. On the other hand, *lacicola* and *montana* originated from *virilis* but not along the line of *novamexicana*,

Table 1. Reciprocal isolation indices among the species groups of *D. melanogaster* and *D. virilis* (*9*).

Female	Male	Isolation index	z (*5*)
melanogaster, simulans	*melanogaster*	0.93	2.87*
melanogaster, simulans	*simulans*	0.76	
melanogaster, mauritiana	*melanogaster*	1.00	5.96*
melanogaster, mauritiana	*mauritiana*	0.62	
simulans, mauritiana	*simulans*	1.00	16.37*
simulans, mauritiana	*mauritiana*	0.12	
virilis, novamexicana	*virilis*	0.97	5.08*
virilis, novamexicana	*novamexicana*	−0.28	
virilis, americana	*virilis*	1.00	5.86*
virilis, americana	*americana*	0.33	
virilis, texana	*virilis*	0.92	4.72*
virilis, texana	*texana*	0.37	
virilis, lacicola	*virilis*	0.97	5.04*
virilis, lacicola	*lacicola*	0.29	
virilis, montana	*virilis*	0.95	3.58*
virilis, montana	*montana*	0.69	
novamexicana, americana	*novamexicana*	0.87	10.19*
novamexicana, americana	*americana*	−0.44	
novamexicana, texana	*novamexicana*	0.80	16.16*
novamexicana, texana	*texana*	−0.15	
novamexicana, lacicola	*novamexicana*	1.00	
novamexicana, lacicola	*lacicola*	1.00	
novamexicana, montana	*novamexicana*	1.00	
novamexicana, montana	*montana*	1.00	
americana, texana	*americana*	0.32	3.75*
americana, texana	*texana*	−0.28	
americana, lacicola	*americana*	1.00	
americana, lacicola	*lacicola*	1.00	
americana, montana	*americana*	1.00	1.02
americana, montana	*montana*	0.97	
texana, lacicola	*texana*	1.00	1.43
texana, lacicola	*lacicola*	0.97	
texana, montana	*texana*	0.97	0
texana, montana	*montana*	0.97	

*Significant at .05 level.

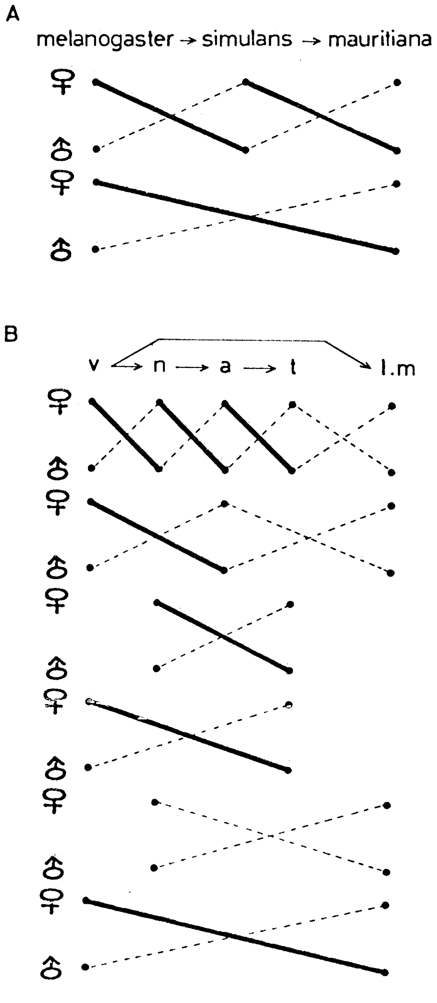

Fig. 1. Schematic relations of mating success and the expected directions of species evolution among the sibling species of (A) *D. melanogaster* and (B) *D. virilis*; (——), more successful mating; (-----), less successful mating. Abbreviations: v, *virilis*; n, *novamexicana*, a, *americana*; t, *texana*; l, *lacicola*; m, *montana*.

americana, and *texana*. The evolutionary process thus obtained in the *virilis* group agrees perfectly with the results of the previous phylogenetic studies.

Mating preference and cytological data indicate the same direction of evolution in one other group of *Drosophila*. On the basis of cytological similarities, Wasserman (*10*) postulated a phylogenetic relationship for the *D. mulleri* subgroup to be *mulleri* → *mojavensis* → *arizonensis*. This is exactly the same order obtained by our method when applied to the data of Patterson (*11*). The phylogeny of *D. willistoni* group has been established from the electrophoretic differences at 36 loci coding for enzymes (*12*). Again, this was almost exactly substantiated by our method when it was applied to the data from two sources (*13, 14*). For sibling species level, the direction of evolution is speculated to be *tropicalis* → *willistoni* → *equinoxialis* → *paulistorum*. For the semispecies level, the evolutionary sequence of *D. paulistorum* is Amazonian → Andean-Brazilian → Orinocan → Interior → Centroamerican → Transitional.

Our hypothesis is useful for understanding the evolutionary sequences of these three groups of *Drosophila* species which originate in North and South America. A newly evolved species would have a small population size and would be exposed to the danger of mixing with the original species. If the newly evolved females mate with the original males, depletion of the new population results. But if a male of the new population mates with a female of the original population, he may mate again with a female of the new population; therefore, the new population is not adversely affected. Genes introduced from the new population into the old will soon be eliminated from the original species by normalizing selection. Thus, the failure of matings between the males of ancestral species and the females of the new species population would be the first step in developing reproductive isolation.

In some Hawaiian *Drosophila* one-side mating preference has been described (*15*). On the basis of ethological examination and the geological history of the islands, Kaneshiro (*2*) postulated a phylogenetic relationship for the *D. planitibia* subgroup to be as follows: *differens* (Molokai) → *planitibia* (Maui) → *heteroneura* and *silvestris* (Hawaii). He assumed that females of derived species mated randomly with males of ancestral species, but that the converse situation did not hold true.

This sequence is exactly reversed if our method is applied to it. Kaneshiro's hypothesis is based on the founder principle of interisland speciation (*16*). A founder individual represents only a portion of the total gene pool of the ancestral population, and the newly derived males have partly lost the courtship pattern of the ancestral species. Therefore, the ancestral females show strong discrimination against males of derived species which lack the total courtship pattern. If our hypothesis is applied to it, a newly derived species would have gained some courtship pattern in addition to the ancestral one. Although there may exist some exceptional cases of degenerative evolution (loss of genetic traits), most new species gain variations that did not exist in the ancestor, as every new trait is obtained in the same manner.

However, our argument is not based on the above assumptions as to whether a newly derived species must have gained or lost some courtship genes in the course of speciation. The most important change for the creation of a new species is the failure of matings between the males of ancestral species and the females of the new species. Many behavioral mechanisms would be involved in the mating failure such that the ancestral males are rejected by the derived females or the derived females are ignored by the ancestral males.

It may be pointed out that the evolutionary sequence given by our hypothesis agrees with the established phylogenies in *Drosophila* groups. Our hypothesis allows a favorable situation for an incipient species to maintain its population and, in fact, indicates a high probability of the new strain accumulating the genetic information necessary for speciation.

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5. Since the test of difference between isolation indices has not yet been established, the difference of the frequency of heterogamic matings was examined. The difference (z) of two proportions was tested by calculating normal deviate:
$$z = (p_1 - p_2)/\sqrt{p_1(1 - p_1)/n_1 + p_2(1 - p_2)/n_2}$$
where p_1 and p_2 were the proportions of heterogamic matings and n_1 and n_2 were the numbers of examined females. The significant difference between the pair of reciprocal matings is accepted at the 5 percent level if the z is greater than 1.96.
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