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Asymmetrical Reproductive Isolation between *Drosophila albomicans* and *D. nasuta*

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Hwei-yu Chang and Yu-Ta Tai (2007) Asymmetrical reproductive isolation between *Drosophila albomicans* and *D. nasuta*. *Zoological Studies* **46**(5): 638-646. The species status of the taxa, *Drosophila albomicans* and *D. nasuta*, has been an issue because no obvious reproductive isolation has been reported. For both *D. albomicans* and *D. nasuta* females, although the mating speeds differed in the single-choice mating tests, without time limitation the successful mating percentage of intraspecific crosses was the same as that of interspecific ones. However, by comparing "multiple-choice", "dual-choice", and "single-choice" experiments, there is evidence for female mating preference and male-male interactions in *D. albomicans*. These 2 components of sexual selection were not observed in *D. nasuta*. The implication of this asymmetrical premating sexual behavior in *D. albomicans* on the speciation history of this species is discussed.
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Key words: Behavior, Evolution, Female choice, Lek, Male interference.

The biological species concept defines species as "groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr 1942). Various reproductive barriers can be categorized as pre- or postmating isolation. Premating isolation mechanisms such as ecological, temporal, behavioral, and mechanical barriers prevent successful mating, while postmating mechanisms may be due to a conflict of the genetic backgrounds on hybrids, such as F_1 inviability, F_1 sterility, and hybrid breakdown (Coyne and Orr 1988, Wu and Palopoli 1994, Wu and Ting 2004). Sexual selection, a major cause of behavioral isolation (Ting 2001), can be classified into female choice and male-male competition. There are male phenotypic traits for female choice (e.g., the number of sex combs, courtship behavior, cuticular hydrocarbons, and body size) in *Drosophila* (Carson 1985, Partridge et al. 1987, Santos et al. 1988, Coyne et al. 1994, Coyne and Charlesworth 1997, Greenspan and Ferveur 2000). Lek behavior, a type of male-male competition, appears in Hawaiian *Drosophila* species (Spieth 1968), several Australia species, and cosmopolitan *Drosophila*. Female choice and male-male competition are important factors in successful mating and may cause reproductive isolation between 2 species.

Drosophila nasuta (Duda 1924) and *D. albomicans* (Lamb 1914) belong to the *D. nasuta* subgroup of the *D. immigrans* species group (Duda 1940, Wilson et al. 1969). The former is found on the eastern coast of tropical Africa, Madagascar, the Seychelles, Sri Lanka, and most of the Indian subcontinent, while the latter is distributed from Southeast Asia to northeastern India, Malaysia, southern China, Taiwan, and Okinawa (southern Japan). According to collection records, no hybrids have ever been found in nature, and Kitagawa et al. (1982) considered them allopatrically distributed species. These 2 sibling species are morphologically identical, but they have differ-

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*To whom correspondence and reprint requests should be addressed. E-mail:hwei@ntu.edu.tw ent karyotypes. The karyotype of *D*. *nasuta* consists of an acrocentric X chromosome, a submetacentric Y, a pair of metacentric 2nd chromosomes, a pair of acrocentric 3rd chromosomes, and a pair of small dot-like 4th chromosomes (Wakahama et al. 1983). The karyotype of *D*. *albomicans* differs from the former in the fusions of the large acrocentric 3rd chromosomes with the X and Y, respectively, such that there are only 3 instead of 4 pairs of chromosomes. Meera Rao and Ranganath (1991) proposed that *D*. *nasuta* ($2n = 8$) retains the ancestral karyotype from which the karyotype of *D*. *albomicans* ($2n = 6$) was derived.

In this study, we used “single-choice”, “dual-choice”, and “multiple-choice” mating experiments to examine mating preferences and behavioral isolation between *D*. *albomicans* and *D*. *nasuta*. Although body size has been reported to influence mating success (Partridge et al. 1987, Santos et al. 1988), our present study indicates that it was not a factor in the *D*. *albomicans* and *D*. *nasuta* mating choice experiments. Comparing results of different mating choice experiments revealed asymmetrical female choice, i.e., *D*. *albomicans* females prefer to mate with conspecific males, whereas *D*. *nasuta* females do not. In addition, *D*. *albomicans* males showed stronger lek behavior than did *D*. *nasuta* males.

MATERIALS AND METHODS

Drosophila strains

Drosophila nasuta strain No. 193.7 from India (established in 1979) and *D*. *albomicans* strain No. 254.29 from Thailand (1993) were used for all experiments including size measurements, 3 kinds of mating choice, and lek behavior observations, throughout this study. Another 3 *D*. *albomicans* strains, No. 254.3 from Thailand (established in 1993), No. 231.3 from Japan (1991), and No. 259.2 from China (1992), were used in the multiple-choice mating experiments. All of them are isofemale lines. They were reared in glass vials (3 cm in diameter, 10 cm high) containing 2.5 cm (depth) of standard corn meal *Drosophila* medium, and placed in an incubator maintained at 23 ± 1°C; with a 12:12 h L:D photoperiod.

Mating choice experiment

Flies were sexed within 8 h after emergence and cultured for 4 d before the experimental manipulation. In total, 400 five-day-old virgin flies (i.e., 200 females and 200 males) were put into a single cage (20 x 20 x 30 cm) for these experiments. Flies used in the dual-choice and multiple-choice mating experiments were marked with fluorescence dust 1 d prior to the experiment. Two colors were adopted (“Aurora Pink” A-11 and “Horizon Blue” A-19, DAY-Glo®, USA). These flies could be identified according to the color under a microscope. Two independent pretests (each with 3 replicas) showed no color preference by *D*. *albomicans* or *D*. *nasuta* females. The 3 kinds of mating choice experiments are described as follows. In general, mated pairs were immediately collected, and the time of forming the pair was recorded within a 1.5 h observation period.

Single-choice mating experiment

Two hundred females of 1 species and 200 males of the same species or 200 males of the other species were placed in a single cage for each experiment. There were 4 combinations of single-choice mating experiments for these 2 species. Under this experimental design, a female had only a single choice: to mate or not to mate.

Dual-choice mating experiment

Two hundred females of 1 species, and 100 males of both species (for a total of 200 males) were placed in a single cage for each experiment. There were 2 combinations in this series of experiments, and a female could choose a mate from among the 2 types of males.

Multiple-choice mating experiment

An equal number (100) of both males and females of these 2 species were placed in a cage for each experiment.

Wing measurement

Wing length was measured as an indicator of body size. From the dual-choice mating experiments, 10 mated and 10 unmated flies of both sexes were randomly chosen. The right wings were dissected and processed through a series of dehydration steps from 70% to 95% ethanol. The specimens were transferred to slides, and embedded in AQUATEX (Merck, Germany), and pictures were taken using a digital camera (Coolpix 4500, Nikon, Japan) under a microscope. A stage micrometer was used to measure wing length (to the nearest 0.01 mm). All data were digitized with tpsDIG32 (ver. 1.40, F. Rohlf, SUNY, Stony Brook, NY, USA) software. The length was measured from the black mark at the base of the radius vein beneath the humeral cross-vein to the end of radius$_{4+5}$ (Fig. 1).

Courtship behavior observations

Behavioral observations were carried out in the morning with 5-d-old virgin flies. These flies were reared under a normal photoperiod (12:12 h L:D) before the day of observation. They were kept in the dark until the preparation was done. Mating behavior within the mating chambers was tape-recorded for 1 h with a Sony DCR-TRV10 DV (Japan), connected to a 40x lens and a white ring light, while some experiments done in vials were recorded directly without taping. Lunge behavior (when a male raises the front part of his body and lunges down onto an opponent) within 30 min in vials was recorded as an indicator of male-male competition.

RESULTS

Body size and mating success

To test whether body size was one of the determining factors of mating success, the wing length was measured in randomly sampled flies from the dual-choice mating experiments. The body size of females was larger than that of males ($p < 0.0001$), but females of these 2 species were the same size ($p = 0.25$). Males of *D. albomicans* were bigger than those of *D. nasuta* ($p < 0.001$). Mated males were about the same size as unmated males (Table 1) in the dual-choice mating experiments with either *D. albomicans* or *D. nasuta*. Since the body size of mated and unmated males was the same, it was excluded as a determining factor of the rate of successful mating in the

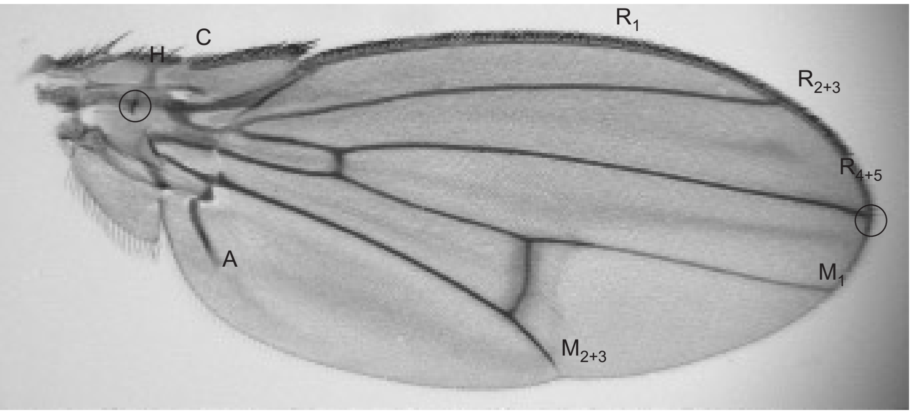

Fig. 1. Wing length index measured as a straight line between the 2 circled points: the black mark at the base of the R (radius) vein near the H (humeral) cross vein and the end of R$_{4+5}$.

Table 1. Wing length measurements (mm, n = 10) of mated and unmated flies in *Drosophila albomicans* and *D. nasuta* dual-choice mating experiments

	Mated	Unmated	*t*-test, *p*
D. albomicans female choice			
a ♀	2.40 ± 0.055	2.43 ± 0.046	0.17
a ♂	2.18 ± 0.024	2.17 ± 0.058	0.8
n ♂	2.11 ± 0.045	2.13 ± 0.057	0.7
D. nasuta female choice			
n ♀	2.39 ± 0.060	2.40 ± 0.044	0.68
a ♂	2.16 ± 0.047	2.17 ± 0.042	0.65
n ♂	2.11 ± 0.052	2.11 ± 0.032	0.45

dual-choice mating experiments with either *D. albomicans* or *D. nasuta*.

Single-choice mating experiment

Intra- and interspecific single-choice mating tests were performed with *D. albomicans* and *D. nasuta*. We pooled the results of mating frequency from 5 cages (each of which contained 200 females and 200 males) because no statistically significant difference existed for the combination. The number of intraspecific *D. albomicans* mating pairs was 395, whereas that of interspecific *D. albomicans* female and *D. nasuta* male pairs was 150. The number of intraspecific *D. nasuta* mating pairs was 532, and that of a paired interspecific *D. nasuta* female and *D. albomicans* male was 531. These results indicated that the successful mating of these 2 species evaluated within 1.5 h differed greatly. *Drosophila nasuta* females showed no preference (χ^2 = 0.001, *d.f.* = 1, p = 0.975) for males of *D. nasuta* or *D. albomicans*, but *D. albomicans* females showed a significant preference for conspecific males (χ^2 = 55.06, *d.f.* = 1, p < 0.00001).

Dual-choice mating experiment

One dual-choice experiment was initiated by putting *D. albomicans* females together with both *D. albomicans* and *D. nasuta* males into a single cage. The other dual-choice experiment used *D. nasuta* females with males of both species. The results of 5 cages for each test were pooled as there were no statistically significant differences at the level of comparison of single cages in either experiment. In the dual-choice mating experiment using *D. albomicans* females, the number of intraspecific mating pairs was 216, while the number of interspecific mating pairs was 81. From this set of data, we determined that *D. albomicans* females preferentially mated with conspecific males (χ^2 = 61.36, *d.f.* = 1, p < 0.0001). There was no difference between this result and that of the single-choice mating experiment (χ^2 = 1.73, *d.f.* = 1, p = 0.18) (Table 2). However, in the *D. nasuta* dual-choice mating experiment, the number of intraspecific mating pairs was 206, while that of interspecific mating pairs was 258 (χ^2 = 5.82, *d.f.* = 1, p < 0.05). This result differed from that for *D. albomicans* females. It showed a greater number of successful mating pairs of *D. nasuta* females with *D. albomicans* males than of *D. nasuta* females with conspecific males. This finding differed from the result of the single-choice mating experiment. Table 2 shows a significant decrease in intraspecific *D. nasuta* mating pairs (χ^2 = 13.33, *d.f.* = 1, p = 0.0005).

Table 2. Comparison of the number of mating pairs from the dual-choice mating experiment with that expected from the single-choice mating experiments

	Expected from single-choice	Observed from dual-choice	χ^2	p
D. albomicans dual-choice				
a♀ x a♂	197.5	216	1.73	0.18
a♀ x n♂	75.0	81	0.48	0.48
D. nasuta dual-choice				
n♀ x a♂	266.0	258	0.24	0.62
n♀ x n♂	265.5	206	13.33	< 0.0005

Multiple-choice mating experiment

There were 4 mating types in a multiple-choice mating experiment performed with both sexes of *D. albomicans* and *D. nasuta*. The data of 5 cages were combined, and the numbers of mating pairs were: 111 for intraspecific *D. albomicans*, 39 for *D. albomicans*-*D. nasuta*, 163 for intraspecific *D. nasuta*, and 228 for *D. nasuta*-*D. albomicans*. The results of the multiple-choice mating experiment showed no significant difference from that expected from the dual-choice mating experiment (Table 3). The expected values were calculated as follows. The total number of *D. albomicans* female mating pairs in the multiple-choice mating experiment (150) multiplied by the ratio of intra- (216/297) and interspecific (81/297) mating pairs in the *D. albomicans* dual-choice mating experiment respectively gave the expected intraspecific *D. albomicans* and interspecific *D. albomicans*-*D. nasuta* values. The total number of *D. nasuta* female mating pairs in the multiple-

Table 3. Comparison of results of the multiple-choice mating experiments with expected data from the dual-choice mating experiment

	Expected from dual-choice	Observed from multiple choice	χ^2	p
a♀ x a♂	109	111	0.13	0.714
a♀ x n♂	41	39		
n♀ x a♂	217	228	1.25	0.26
n♀ x n♂	174	163		

choice mating experiment (391) multiplied by the ratio of intra- (206/464) and interspecific (258/464) mating pairs in the *D*. *nasuta* dual-choice mating experiment respectively gave the expected intraspecific *D*. *nasuta* and interspecific *D*. *nasuta*-*D*. *albomicans* values.

Multiple-choice tests were also performed using *D*. *albomicans* strains from other geographic regions. The results were compared with those using strain No. 254.29, and no significant differences were found (Table 4). Moreover, regardless of the type of mating experiment, our results consistently showed low mating rates for *D*. *albomicans* females and *D*. *nasuta* males.

Mating speed

In the single-choice mating experiment, the number of mating pairs was recorded every 10 min. Figure 2 shows the accumulated intraspecific mating pairs of *D*. *nasuta* and *D*. *albomicans* within 90 min. These results indicated that the mating speed of *D*. *albomicans* females was slower than that of *D*. *nasuta* females. Figure 3 shows the numbers of intra- and interspecific mating pairs of *D*. *nasuta* females, indicating that the speed was nearly the same.

Courtship behavior

There were 2 experimental designs for mating chamber observations: one with 6 males in a chamber, and the other with 6 males plus 2 *D*. *albomicans* females. In total, 12 one hour tapes including 6 "males only", and 6 "males with females" consistently showed strong male-male interactions among *D*. *albomicans* males in the presence of females. No interference behavior, such as wing erect, tussle, lunge, chasing, etc., was observed in *D*. *albomicans* males when no females were present. No such behavior was found in *D*. *nasuta* males regardless of the presence or absence of females.

In the vial observations without tape recording, 5 females were placed into a vial (3 cm in diameter and 10 cm high) together with either 10 *D*. *albomicans* males or 10 *D*. *nasuta* males, and were concentrated into a small space (4 cm high)

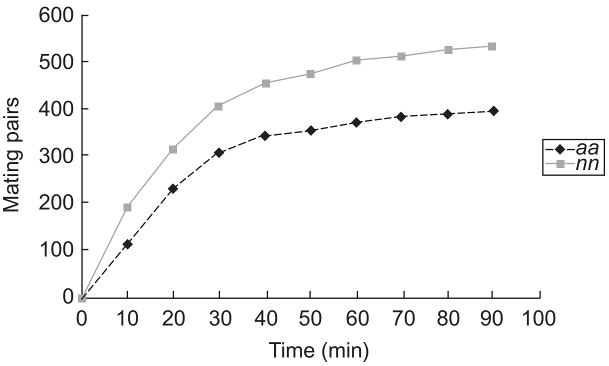

Fig. 2. Cumulative number of mating pairs each 10 min within 90 min in 2 types of "single-choice" experiment (with data from a total of 5 experiments). The squares (*nn*) indicate *Drosophila nasuta* mating pairs; the diamonds (*aa*) indicate *D*. *albomicans* mating pairs.

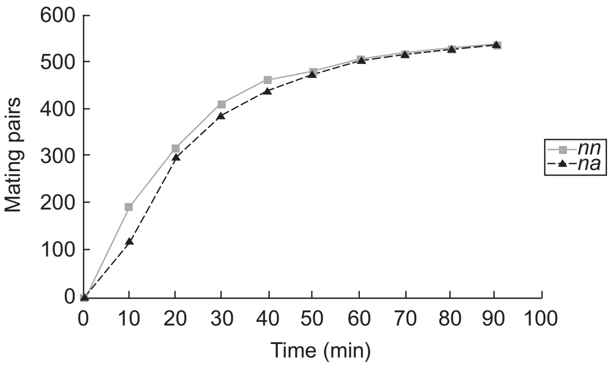

Fig. 3. Cumulative number of mating pairs each 10 min within 90 min in 2 types of "single-choice" experiment (with data for a total of 5 experiments). The squares (*nn*) indicate *Drosophila nasuta* mating pairs; the triangles (*na*) indicate *D*. *albomicans* female and *D*. *nasuta* male mating pairs.

Table 4. Number of mating pairs from the multiple-choice mating experiments using different *Drosophila albomicans* strains. The χ^2 test was based on the expected values weighted according to the number of replicas from the results when using strain No. 254.29

Strain	No. of replications	a♀ x a♂	a♀ x n♂	n♀ x n♂	n♀ x a♂	χ^2	*p*
254.3	4	86	24	139	149	0.50	0.47
231.3	3	64	29	111	124	1.50	0.21
259.2	3	66	26	100	143	0.01	0.89

by pushing the cotton plug. Lunge behavior (i.e., typical lek behavior, in which a male bumps another male with its head upon an encounter) within 30 min was recorded. Summing up 9 replicates, in the presence of *D. albomicans* females, 75 lunges were observed in *D. albomicans* males but only 5 in *D. nasuta* males. Comparing 5 *D. nasuta* females and 10 *D. nasuta* males was not informative, because courtship behavior ceased after the females had copulated, and in 8 of 9 replicates, all females had formed mating pairs within 20 min.

DISCUSSION

The single-choice mating experiments indicated that *Drosophila albomicans* females took much more time to accept mates than did *D. nasuta* females. More mating pairs formed given a longer observation time, but differences in mating propensity may have confounded the results. According to the 50% criterion recommended by Casares et al. (1998), the cage experiments were recorded for 90 min. Taking the successful mating pairs formed in 1.5 h as an indicator of choosiness, the number of intraspecific pairs of *D. albomicans* was much lower than that of *D. nasuta*. The data also showed that *D. albomicans* females preferred to mate with *D. albomicans* males, but *D. nasuta* females had no preference. The preference of *D. albomicans* females to conspecific males was consistent between the dual-choice and multiple-choice experiments. In the dual-choice and multiple-choice mating experiments, successfully mated pairs that formed between *D. albomicans* females and *D. nasuta* males outnumbered intraspecific *D. nasuta* pairs. The results of multiple-choice mating experiments were also informative. If mating pairs (Table 3) are indicators of preference, females of both species preferred *D. albomicans* males, although the number of mating pairs of *D. albomicans* females was lower than that of *D. nasuta* females. This phenomenon was consistent when different *D. albomicans* strains from Japan (No. 231.3), Thailand (No. 254.3), and China (No. 259.2) (Table 4) were used in the multiple-choice mating experiments.

Females might choose a recognizable characteristic, but these 2 species are nearly morphologically identical, with only slight differences in body size. A larger body size of *D. albomicans* than *D. nasuta* was also reported by Harini and Ramachandra (2003). Body size is a fitness-related phenotype (Ruiz et al. 1991). Thorax length and abdomen length can be used as indicators of body size (Partridge et al. 1987, Santos et al. 1988 1992), but wing length (Sokoloff 1966) is better in that it is less affected by the environment after eclosion. Moreover, measurement of a flat wing showed higher precision than the other indicators. Males of *D. malerkotliana* with a bigger body size had superior mating ability (Hegde and Krishna 1997). Although this is not universally true in *Drosophila*, for instance, smaller males in *D. subobscura* had a higher courtship advantage (Steel and Partridge 1988), in many species larger males have been demonstrated to exhibit mating advantages. Partridge et al. (1987) found that mated males of *D. melanogaster* were bigger than those which remained unmated. Santos et al. (1988) showed that bigger males in *D. buzzatii* had a higher chance of being chosen by females for mating. Body size may influence mating time, reproductive ability, and other fitness characters (Ewing 1961, Partridge and Farquhar 1981 1983, Ruiz and Santos 1989).

Under our culture conditions, females of both species were larger than males, which is consistent with a previous study (Harini and Ramachandra 2003). In the dual-choice mating experiments, mated females were about the same size as unmated females, which is consistent with results of Partridge et al. (1987). Whether mated or unmated, males of *D. albomicans* were larger than those of *D. nasuta* (Table 1). Although the interspecific difference in males was significant, body size had no influence on intraspecific mating success in either *D. albomicans* or *D. nasuta*. Therefore, the hypothesis that females mate with larger males was rejected in the context of this study.

In the single-choice mating experiment, male-male interactions did not exist, while in the dual-choice and multiple-choice mating experiments, successful mating was influenced not only by females but also by male-male interactions. The 2 influencing components could be partitioned by comparing the single-choice with the dual-choice mating experiments. In the dual-choice mating experiments, the total of the 2 species of males was 200, but in the single-choice experiments 200 males were all of 1 species. Therefore we calculated the expected numbers for dual-choice mating experiments by dividing the appropriate number by the data of the single-choice experiments. In the *D. nasuta* dual-choice mating experiment, the incidence of intraspecific mating pairs was significantly lower than expected (Table 2). We suggest that interactions between the 2 species of males were disadvantageous for *D. nasuta* males. However, male-male interactions were not apparent in *D. albomicans* dual-choice mating experiments. Since *D. albomicans* females do not prefer *D. nasuta* males and their mating speed is slow, there were too few successful matings to detect an effect of male-male interactions.

Drosophila albomicans and *D. nasuta* easily produce hybrids in the laboratory, and a hybrid population can be maintained for hundreds of generations (Tanuja et al. 1999). These 2 species have different karyotypes, and karyotype polymorphism persists after long-term cultivation of hybrid populations (Yu et al. 1997). Nevertheless, no hybrid karyotype has ever been found in any of the strains of these 2 species we examined. This evidence supports the hypothesis that they are allopatrically distributed. In a previous study, we did not find much reproductive isolation other than hybrid breakdown (Chang and Ayala 1989). By designing these cage experiments, our data showed asymmetrical female choice and male-male competition in *D. albomicans*.

In the multiple-choice mating experiments, both female choice and male-male competition existed as in the dual-choice mating experiments. To examine the data from the multiple-choice mating experiments again from this point of view, we partitioned the multiple-choice data into 2 sets of dual-choice data, and took into consideration the mating speed in experiments of *D. albomicans* females that have a slower mating speed (Fig. 2). In contrast, *D. nasuta* females mated with either male at similar speeds (Fig. 3). Therefore, the expected number of mating pairs was weighted according to the differential mating speeds. The results of the multiple-choice mating experiments did not differ from what was expected (Table 3). Without this careful analysis, the results of the multiple-choice mating experiment might have mistakenly generated the hypothesis that both *D. albomicans* and *D. nasuta* females prefer *D. albomicans* males. Not only dual-choice but also multiple-choice mating experiments supported an alternative hypothesis that *D. albomicans* females prefer conspecific males and that *D. albomicans* males tend to win against *D. nasuta* males during male-male interactions.

Lek behavior has been observed in some Hawaiian *Drosophila* species (Spieth 1974, Droney 1992, Hoikkala and Welbergen 1995). This behavior represents competition between males, which influences both mating success (Droney 1992. Hoikkala and Welbergen 1995) and mating speed. Videotapes showed strong lek behaviors of *D. albomicans* males, including wing erect, tussle, and lunge (Hoffmann 1987). Lunge was used as an indictor of lek behavior as its frequency was much higher than wing erect, and it is much easier to record than tussle or other behaviors. Lek behaviors were only observed in the presence of virgin females and ceased after females had been mated. No lunge behavior was recorded in the intraspecific *D. nasuta* sets probably because in eight of 9 replicates all 5 females had copulated within 20 min. Since the mating speed of *D. albomicans* females was slower (Fig. 2) than that of *D. nasuta*, virgin females existed during the 30-min observation period. In the presence of virgin *D. albomicans* females, the lunge behavior of *D. albomicans* males was counted 75 times versus only 5 times for *D. nasuta* males.

Some studies have indicated that there is no correlation between asymmetrical mate choice and polarity of mating behavior (Wasserman and Koepfer 1980, Moodie 1982, Koepfer 1991), except that Kaneshiro (1976) and Watanabe and Kawanishi (1979) proposed the opposite. There are 2 hypotheses with respect to asymmetrical reproductive isolation. One states that ancestral females prefer to mate with conspecific males (Kaneshiro 1976); the other proposes that derived females prefer to mate with conspecific males (Watanabe and Kawanishi 1979). These 2 hypotheses may refer to different sets of *Drosophila* species. Kaneshiro (1976) observed asymmetrical mate choice in Hawaiian *Drosophila*, with the polarity of mating behavior being inferred according to the ages of the islands. Since the islands were isolated by the ocean, speciation was promoted by the existence of this geographical barrier. Kaneshiro concluded that ancestral females prefer conspecific males, but derived females do not. Interestingly, Watanabe and Kawanishi (1979) found that in species evolved with overlapping ranges, derived females prefer conspecific males, but ancestral females do not. The speciation mechanism of species with an effective geographical barrier might differ from that of species lacking such a barrier. The former might have involved losing some courtship elements through genetic drift or adaptation to a different environment (Kaneshiro 1976 1980), which explains the rejection of a derived male by an ancestral female. In the latter case without an effective geographical barrier, if derived females reject original males, then the derived population might have a higher chance of differentiating from the original population. This might be related to Watanabe and Kawanishi's model. According to karyotypic data, *D. albomicans* is derived. The direction of this asymmetrical mating choice therefore fits Watanabe and Kawanishi's model. These derived males may have evolved new male performances instead of having lost some courtship elements. This implies that at the beginning of speciation, no effective gene flow barrier existed.

In *D. albomicans*, we found significant female preference and at the same time an indication of male lek behavior. Roughly estimated from salivary gland chromosomes, the X chromosome is about 20% and the 3rd chromosome 40% of the genome (Lin et al. 1974). Having 60% of the sex-linked genome through fusion events of autosomes and sex chromosomes (X and Y separately) might increase the possibility of evolving new male-specific traits. The results of our dual-choice and multiple-choice experiments revealed an advantage for *D. albomicans* males in the lek behavior. The lek behavior of *D. albomicans* males may have been disadvantageous in the ancestral population unless it had coevolved with female discrimination in a small peripheral population. *D. albomicans* might have originated from such a population, differentiated, and then expanded to its current distribution. This incomplete asymmetrical reproductive isolation might therefore not be a mechanism that was reinforced (Dobzhansky 1951) after the populations differentiated, but instead might have been an important factor during divergence.

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