Mating Asymmetries and Phylogeny in the *Drosophila melanogaster* Species Complex[1]

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ABSTRACT: The propensities for interspecific courtship and mating among the four species of the *Drosophila melanogaster* species complex were studied to examine Kaneshiro's hypothesis relating asymmetrical interspecific mating to the direction of phylogeny. Strong asymmetries were revealed, especially involving the ability of *sechellia* and *mauritiana* males to inseminate *simulans* females. A possible basis for these asymmetries involving partial mechanical isolation is proposed. The relationship to phylogeny, if any, remains unclear.

USING THE INDEPENDENTLY INFERRED PHYLOGENY of several Hawaiian *Drosophila*, Kaneshiro (1976, 1983) has suggested that the well-known asymmetrical propensities for interspecific mating in *Drosophila* and other organisms may reflect the direction of phylogeny. He hypothesized that females of derived species are more likely to mate with males of ancestral species than vice-versa because of a loss in complexity in the courtship of the derived species. This potentially powerful hypothesis has now been subjected to several tests and refinement, but the actual behavioral basis for the asymmetries is not known for any example, and the relationship to phylogeny is not yet generally accepted (for a review, see Giddings and Templeton 1983).

The *Drosophila melanogaster* species complex has been used as an example supposedly conflicting with Kaneshiro's model (Watanabe and Kawanishi 1979). An earlier analysis of the three species then known in the complex did not resolve the question (Robertson 1983a), but now a fourth species, *D. sechellia*, has been discovered (Tsacas and Bachli 1981). It is very closely related to *simulans* and *mauritiana* (Lemeunier and Ashburner

1984, Lachaise et al. 1986). Here I describe the propensities for interspecific courtship and mating for the four species now known, suggest a novel basis for the asymmetries observed, and discuss possible relationships to phylogeny.

METHODS

The tests were conducted in a "no-choice" fashion by placing ten females and ten males together in a yeasted food vial and monitoring the number of courtships and copulations in progress each minute for 10 min. The vial was then set aside for 48 hr, after which the females were placed individually in food vials that were checked for the presence of hybrid progeny after 14 days. Hybrid males can be recognized by the intermediate shapes of their genitalia (Robertson 1982, Coyne 1983). Five and sometimes ten replicates were conducted for each possible pairing of the four species. The entire experiment was repeated using males whose foretarsi (but not the basal tarsal segment carrying the sex combs) had been surgically removed under ether anesthesia. Other methods were similar to those used earlier (Robertson 1983a), except that the *sechellia* female virgins were collected without ether anesthesia by aspiration because preliminary tests showed that ether had a sterilizing effect on them. Stocks were isofemale lines recently derived from the wild. M. Picker collected *mauritiana* in

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TABLE 1

PROPENSITIES FOR COURTSHIP AND MATING IN THE *Drosophila melanogaster* SPECIES COMPLEX

PAIRING		NO. OF REPLICATES	PERCENT COURTING EACH MINUTE	PERCENT MATING IN 10 MIN	PERCENT MATING IN 48 HR
♂	♀				
mel	mel	5 (5)	64 (19)	72 (28)	98 (96)
mel	sim	5 (5)	10 (0)	0 (0)	2 (4)
mel	mau	10 (5)	0 (0)	0 (0)	0 (0)
mel	sec	10 (10)	80 (24)	0 (0)	4 (7)
sim	sim	5 (5)	39 (8)	44 (6)	98 (92)
sim	mel	5 (5)	0 (2)	0 (0)	0 (0)
sim	mau	5 (5)	27 (3)	0 (0)	0 (0)
sim	sec	10 (10)	1 (0)	0 (0)	0 (0)
mau	mau	5 (5)	73 (44)	78 (32)	98 (94)
mau	mel	5 (5)	1 (4)	0 (0)	4 (32)
mau	sim	5 (5)	28 (18)	32 (20)	76 (96)
mau	sec	10 (10)	0 (0)	0 (0)	0 (0)
sec	sec	5 (5)	36 (6)	34 (0)	98 (96)
sec	mel	5 (5)	3 (1)	0 (0)	12 (2)
sec	sim	5 (5)	19 (5)	8 (0)	48 (18)
sec	mau	10 (5)	0 (0)	0 (0)	0 (0)

NOTE: mel = *melanogaster*, sim = *simulans*, mau = *mauritiana*, sec = *sechellia*; figures in parentheses are for foretarsiless males.
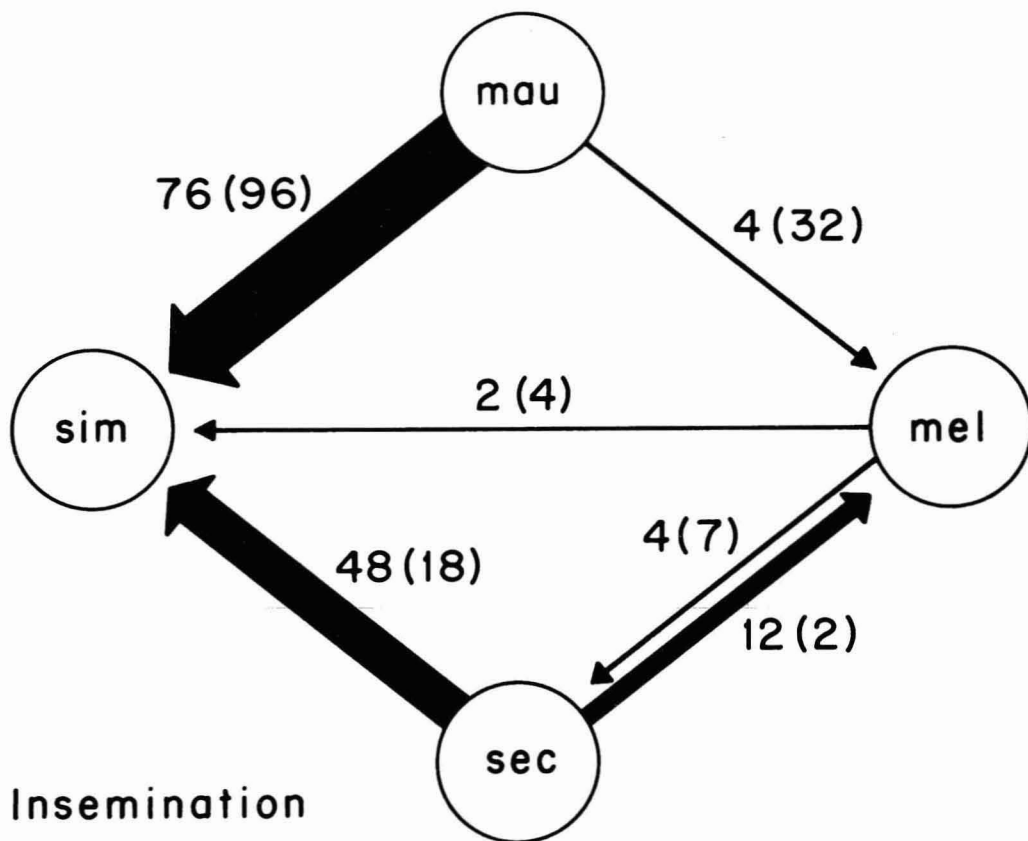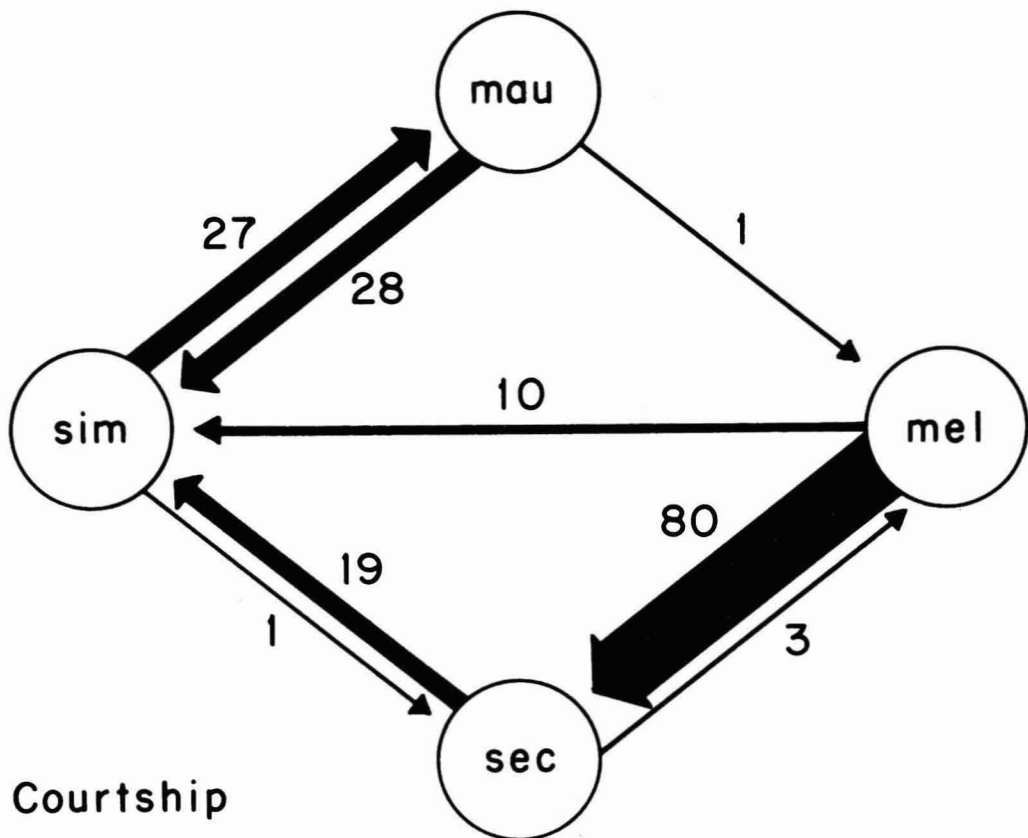
1979, L. Tsacas found *sechellia* in 1980, and T. Lyttle provided *simulans* and *melanogaster* from Hawaii in 1982.

COURTSHIP PROPENSITIES

The propensities for interspecific courtship are presented in Table 1 and diagrammed in Figure 1. The results for *melanogaster*, *simulans*, and *mauritiana* are reasonably consistent with results reported elsewhere for these species by myself and others. Most studies have found that *melanogaster* males court *simulans* females more vigorously than reported here (e.g., Manning 1959, Wood and Ringo 1980, Kawanishi and Watanabe 1981, Robertson 1983a). They all used long-established laboratory strains of *melanogaster*, however, and it is possible that males of the recently wild-caught strain used here are more species specific in their responses. Similarly specific responses were reported by Schilcher and Dow (1977), but they do not report the source of their strains. In addition, the *simulans* strains used in these experiments are from varied locations, and Luyten (1982) has reported that *simulans* strains from

various parts of Africa vary in the cuticular hydrocarbons that elicit male courtship, so we might expect to find differences in the propensities of nonconspecific males to court *simulans* females of different strains. Males of the *melanogaster* strain used here were also less likely to court *mauritiana* females than were the Canton Special males used earlier (Robertson 1983a), a result also consistent with those of Schilcher and Dow (1977). While these strain differences are disconcerting, major asymmetries are nevertheless consistent across studies. These intraspecific strain differences may yet prove to be highly informative. The *simulans* differences, taken together with differences found by Lachaise et al. (1986) in hybrid gonadal development of various *simulans* strains when crossed to *mauritiana* or *sechellia*, may be revealing ongoing differentiation and speciation in Africa. The *melanogaster* differences may allow more refined genetic analysis of these behaviors.

The results for *sechellia* show particularly strong asymmetries. Specifically, the courtship of *sechellia* females by *melanogaster* males was extremely vigorous, perhaps even more so than in intraspecific pairings. These

Courtship

Insemination

results are partially explicable by the finding that *melanogaster* and *sechellia* share a similar profile of cuticular hydrocarbons, and that it is different from that shared by *simulans* and *mauritiana* (Luyten 1982). The asymmetries nevertheless remain unexplained because we do not yet understand precisely which chemicals are important as pheromones.

MATING PROPENSITIES

The propensities for interspecific mating also are presented in Table 1 and Figure 1. Again, the results differ somewhat from those obtained earlier, presumably for similar reasons. Inclusion of *sechellia* emphasizes the often neglected point that male courtship and female receptivity are two separate aspects of interspecific mating. For example, the intense courtship of *sechellia* females by *melanogaster* males seldom led to insemination. Unfortunately, these two aspects seldom can be so easily separated, because if the males do not court at all (as in the pairing of *sechellia* and *mauritiana*), potential asymmetries in the female propensities for interspecific mating cannot be examined. This problem cannot be circumvented completely, because in the absence of all chemical stimuli, produced by complete removal of the males' foretarsi and antennae, males will not court at all (Robertson 1983*b*). Removal of the foretarsi alone can reduce the specificity of male courtship propensities somewhat (Manning 1959, Robertson 1983*a*), so the experiment was repeated with foretarsiless males. While seldom increasing courtship in 10 min, this operation can enhance interspecific mating, most notably in the pairing of *mauritiana* males and *melanogaster* females (Table 1, Figure 1). Nevertheless, some doubt must remain about the interspecific mating propensities of females when males do not court vigorously.

MATING ASYMMETRIES

I suggested earlier (Robertson 1983*a*) that the asymmetrical ability of *mauritiana* males to inseminate *simulans* and, to a lesser extent, *melanogaster* females might be explained by the remarkably swift attempts at copulation made by the *mauritiana* males, which regularly resulted in copulations before the females could resist. This phenomenon was again conspicuous in watching flies in the initial 10 min (Table 1). In the other pairing showing strong asymmetry, *sechellia* males were able to copulate with *simulans* females. There was no indication that a similar explanation would apply here, however, because the males' attempts at copulation were no swifter than those of *melanogaster* or *simulans* males, and the females did not resist the copulations as they do with *mauritiana* males (Robertson 1983*a*).

I therefore suggest another explanation for these asymmetries: partial mechanical isolation caused by the different shapes of the posterior processes (lobes) on the males' genital arches. The shape of this process is the major taxonomic character used to distinguish the males of these species. The genetic basis for the species differences is even known to be polygenic with a strong autosomal component and little dominance (Robertson 1982, Coyne 1983). The process varies enormously in size from a large clam-shaped plate in *simulans* to a narrow peg in *mauritiana* (Figure 2). During copulation it is used as a clasper on the outside of the female's abdomen and is inserted under the 8th and 9th tergites, where they overlap at the base of the 9th segment (Figure 3). This point of insertion is largest in *simulans* females, presumably to accommodate the large processes of their males (Figure 2). Hence, it is conceivable that the overall asymmetries result from the mechanical inability of males to copulate with females of species whose males have smaller posterior processes. The

FIGURE 1. Propensities for interspecific courtship and mating in the *Drosophila melanogaster* species complex. Propensities for interspecific courtship are indicated by the mean percentage of pairs courting or copulating each minute for the first 10 min of the experiment. Interspecific mating is indicated by the percentage of females inseminated in 48 hr. The values in parentheses are for foretarsiless males. The arrows point from male to female.
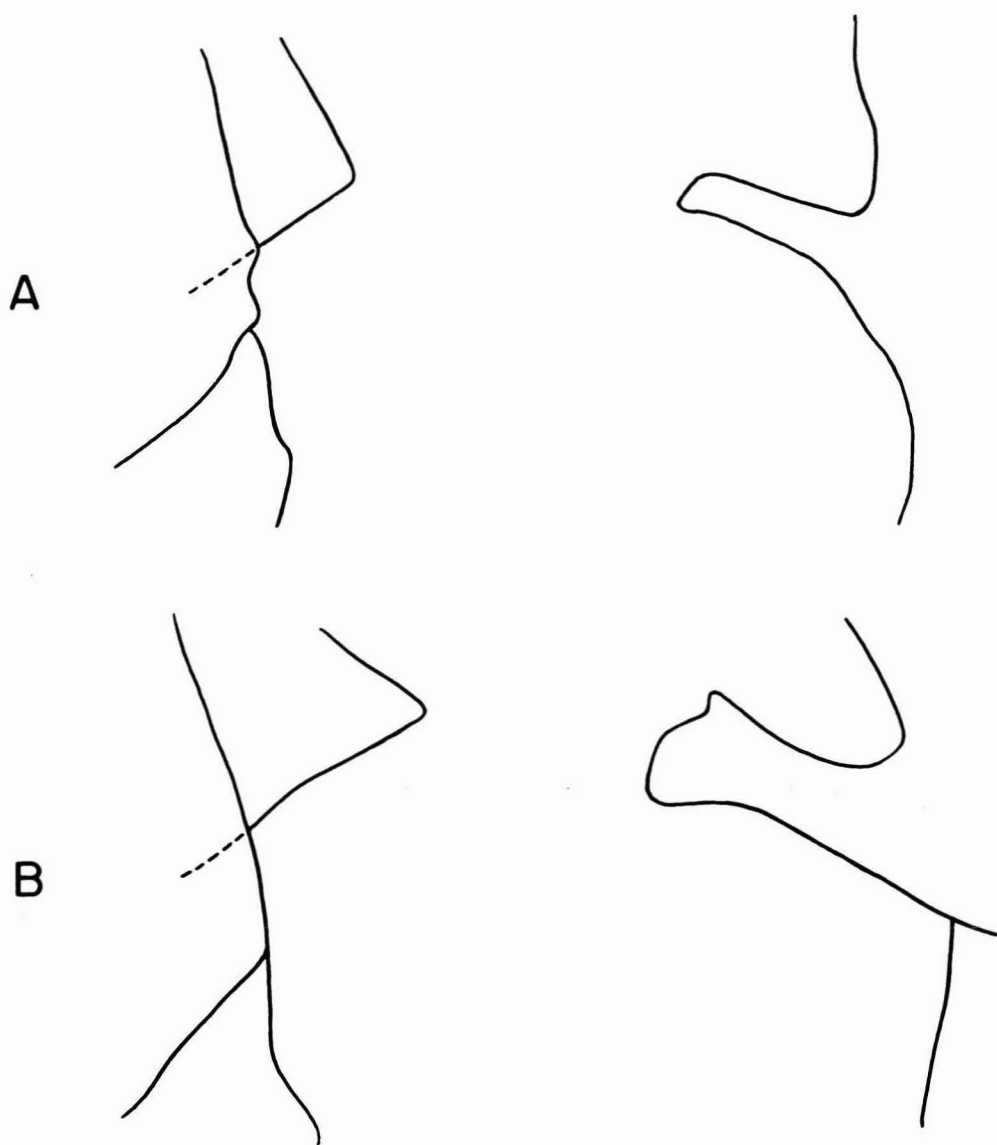
FIGURE 2. Shapes of the female 8th and 9th abdominal tergites (left) and the posterior processes of the male genital arches (right) in the *Drosophila melanogaster* species complex. The drawings were traced from scanning electron micrographs such as those shown in Figure 3. The female tergites are shown in lateral view, dorsal above and posterior to the right. The male processes are also in lateral view but inverted, dorsal below and posterior to the left, as they are during copulation (see Figure 3). Magnification is approximately ×400. A, *mauritiana*; B, *sechellia*; C, *melanogaster*; D, *simulans*.

extreme mating asymmetries involve the species with the largest differences in the size of the processes, i.e., *simulans–mauritiana* and *simulans–sechellia*. Similar asymmetries are observed in a well-documented case of mechanical isolation, damselflies of the genus *Enallagma* (Paulson 1974). In the *Enallagma* damselflies, the morphological differences appear to be of importance primarily in tactile mate recognition, with the mecha-
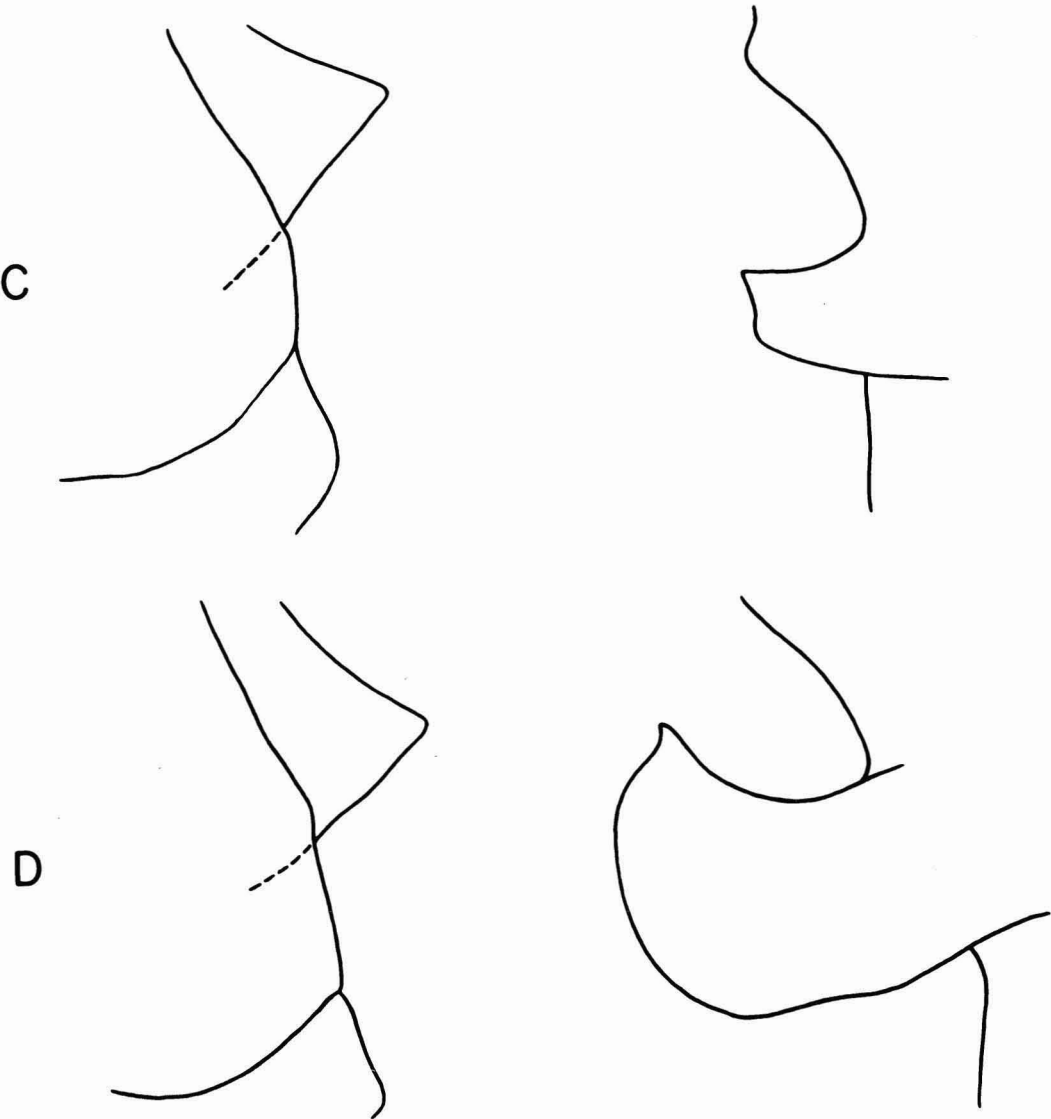
FIGURE 2 (continued)

nical isolation being an incidental side effect (Robertson and Paterson 1982), a thesis worth considering for these *Drosophila*. This explanation of the asymmetries in terms of partial mechanical isolation could be tested if we could remove the posterior processes from *simulans* males (which should then be more able to inseminate *mauritiana* females), but attempts to remove completely this tiny structure using microsurgery and opthalmic surgical lasers have failed. Mechanical isolation has not proved to be a common phenomenon, largely because differences in mate recognition systems usually prevent interspecific interactions from reaching the stage of genital contact. If confirmed, this example would therefore be rather unusual.

Kaneshiro's (1976) explanation of the behavioral basis for mating asymmetries involved loss of elements or complexity from
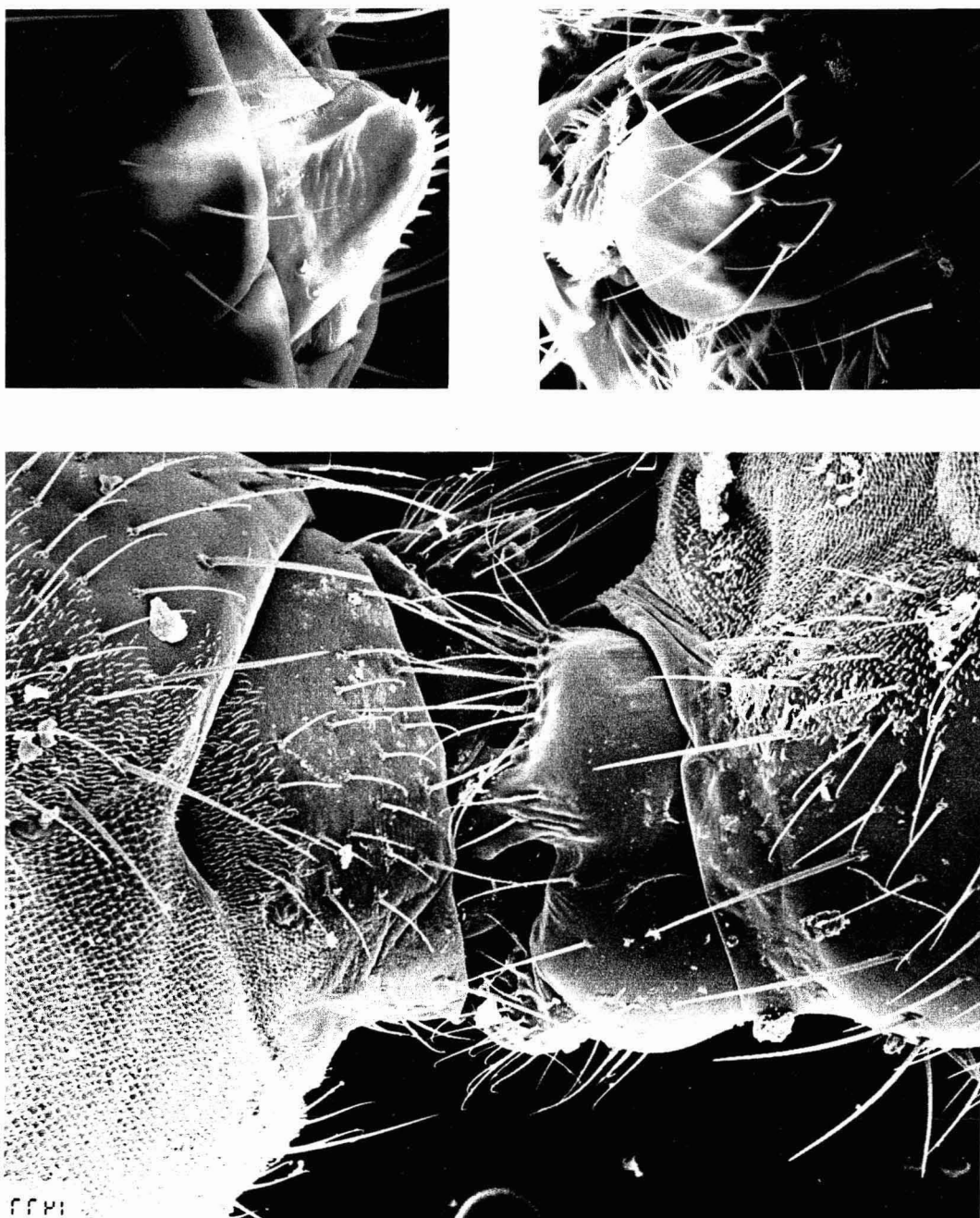
FIGURE 3. Scanning electron micrographs of the genitalia and genital contact during copulation of *Drosophila simulans*. The genital contact (bottom) was obtained by freezing copulating pairs in liquid nitrogen. The female is on the left. The genitalia (top) are oriented as in Figure 2. Magnification is approximately × 400.

the mate recognition system of the derived species during founder events. Giddings and Templeton (1983) added the possibility of sexual selection mediating these changes, and Lambert (1984) has suggested various ways in which such changes in mate recognition systems may cause the asymmetries. I have found no difference in the complexity of mate recognition between *simulans* and *mauritiana* (Robertson 1983*a*), and superficial examination reveals none between *sechellia* and these species. The only potential example in the literature involves instead the addition of a row of bristles on the foretarsi of males of certain populations of *D. silvestris* on the island of Hawaii. The effect of this addition is unclear. Spiess and Carson (1981) found that it enhanced mating between the ancestral females and derived males, but in the data of Kaneshiro and Kurihara (1981) it seems to have little effect, and they interpret the situation as derived females preferring ancestral males. So there is little support for Kaneshiro's explanation of the behavioral basis for the asymmetries, but the paucity of detailed observational and experimental studies of mating behavior preclude any final evaluation.

PHYLOGENY

The genetic relationships of these species are fairly well understood. *Drosophila mauritiana*, *simulans*, and *sechellia* appear equally closely related to each other, with homosequential chromosome banding sequences (Lemeunier and Ashburner 1984) and sterile male but fertile female hybrids (Lachaise et al. 1986). A large body of genetic evidence shows that *melanogaster* is more distantly related to these species (see references in Robertson 1983*a*, Lemeunier and Ashburner 1984, Lachaise et al. 1986).

These species are joined in the *melanogaster* subgroup by another four African species in the *yakuba* complex, and their biogeography is currently under study (Tsacas 1984). The subgroup is endemic to the Afrotropical region but originated from India, allowing two alternative phylogenetic scenarios. First, an old invasion of Africa led to the evolution there of both species complexes, with *sechellia* and *mauritiana* evolving relatively recently from colonizations by *simulans* of the islands of the Seychelles and Mauritius, respectively, in the Indian Ocean. Second, either *sechellia* or *mauritiana* represent the original migrants from India and led to further invasion of Africa, and most recently to the evolution of *simulans* and *mauritiana* or *sechellia*. There is as yet no compelling evidence for deciding between these scenarios. Kaneshiro's hypothesis predicts that the latter is correct, while those studying the complex are disposed to the former (Lemeunier and Ashburner 1984, Tsacas 1984). Considering the apparently complex behavioral basis for the asymmetries in these species, involving asymmetrical male courtship propensities, different speeds of male attempts at copulation, partial mechanical isolation, and probably still other asymmetrical differences in their mate recognition systems, I doubt that there is any simple instructive relationship to phylogeny in this group.

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