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SEXUAL ISOLATION BETWEEN TWO SIBLING SPECIES WITH OVERLAPPING RANGES: *DROSOPHILA SANTOMEA* AND *DROSOPHILA YAKUBA*

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Abstract.—*Drosophila yakuba* is widespread in Africa, whereas *D. santomea*, its newly discovered sister species, is endemic to the volcanic island of São Tomé in the Gulf of Guinea. *Drosophila santomea* probably formed after colonization of the island by a *D. yakuba*–like ancestor. The species presently have overlapping ranges on the mountain Pico do São Tomé, with some hybridization occurring in this region. Sexual isolation between the species is uniformly high regardless of the source of the populations, and, as in many pairs of *Drosophila* species, is asymmetrical, so that hybridizations occur much more readily in one direction than the other. Despite the fact that these species meet many of the conditions required for the evolution of reinforcement (the elevation of sexual isolation by natural selection to avoid maladaptive interspecific hybridization), there is no evidence that sexual isolation between the species is highest in the zone of overlap. Sexual isolation is due to evolutionary changes in both female preference for heterospecific males and in the vigor with which males court heterospecific females. Heterospecific matings are also slower to take place than are homospecific matings, constituting another possible form of reproductive isolation. Genetic studies show that, when tested with females of either species, male hybrids having a *D. santomea* X chromosome mate much less frequently with females of either species than do males having a *D. yakuba* X chromosome, suggesting that the interaction between the *D. santomea* X chromosome and the *D. yakuba* genome causes behavioral sterility. Hybrid F_1 females mate readily with males of either species, so that sexual isolation in this sex is completely recessive, a phenomenon seen in other *Drosophila* species. There has also been significant evolutionary change in the duration of copulation between these species; this difference involves genetic changes in both sexes, with at least two genes responsible in males and at least one in females.

Key words.—Mate discrimination, reinforcement, reproductive isolation, sexual isolation, speciation.

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Although sexual isolation is likely to be one of the earliest evolving and most important isolating barriers in animals, characterizing this isolation, both phenomenologically and genetically, is difficult. Most animal species are difficult to study in the laboratory, and genetic analysis can be performed only on species having relatively short generation times and well-characterized genetic markers. For this reason most such studies, especially those involving genetics, have been limited to *Drosophila*, particularly three species in the *D. simulans* subgroup: *D. simulans*, *D. mauritiana*, and *D. sechellia* (see table 1 of Coyne and Orr 1998) and African races within the cosmopolitan outgroup *D. melanogaster* (Ting et al. 2001).

Unfortunately, species in the *D. simulans* subgroup are allopatric: *D. simulans* is a worldwide human commensal, whereas *D. mauritiana* and *D. sechellia* are island endemics, restricted respectively to Mauritius and the Seychelles. One would like to study sexual isolation between taxa living in the same place, for it is only those taxa that can confidently be considered good biological species. Moreover, one of the most intriguing questions about speciation—that of reinforcement, the view that natural selection can increase sexual isolation between sympatric taxa by favoring individuals who do not produce maladapted hybrids—can be studied only in species that are at least partially sympatric (for a history of the concept of reinforcement, a summary of the evidence supporting it, and an evaluation of its present importance, see Howard 1993; Noor 1999). As Howard and Noor note, a recent revival of interest in reinforcement has been based on empirical studies showing dramatically higher sexual isolation between sympatric than between allopatric species (Coyne and Orr 1989, 1997) and on the development of realistic theoretical models showing that reinforcement might occur with respectable frequency (Liou and Price 1994; Kelly and Noor 1996; Kirkpatrick 2000; Servedio 2000).

Recent field studies have uncovered a pair of sister species in the *D. melanogaster* group, *D. yakuba* and *D. santomea* (Lachaise et al. 2000) that have overlapping ranges and form hybrids in the area of overlap. As the only species in this group that are partially interfertile and also partially sympatric, this pair offers the chance to test the generalities about sexual isolation drawn from other species in the group and to determine whether, as predicted by the theory of reinforcement, sexual isolation between the species is highest in the area of overlap.

Drosophila yakuba is widespread across sub-Saharan Africa and on the islands near the continent, but *D. santomea*, discovered in 1998, is endemic to São Tomé, an 860-km^2 volcanic island 320 km west of Gabon (Lachaise et al. 2000). *Drosophila yakuba* also lives on São Tomé. On the mountain Pico do São Tomé, *D. yakuba* is limited to elevations below 1450 m, and *D. santomea* lives in the mist forests at elevations between 1153 m and 1600 m. No attempts have been made to collect *D. santomea* above this elevation: it may exist up to the mountain peak at 2024 m. *Drosophila yakuba* also lives at other lowland sites on São Tomé.

Between 1100 m and 1450 m elevation on Pico do São Tomé, the species' distributions overlap, with the ratio of *D. yakuba*/*D. santomea* shifting from 2.0 to 0.05 as one moves higher through the zone. The species show some sexual isolation when tested in the laboratory (Lachaise et al. 2000), and there appears to be a low frequency (~1%) of hybrids in the zone of overlap using morphological criteria (*D. yakuba* has the black abdominal pigmentation typical of all other species in the *D. melanogaster* group, whereas *D. santomea* lacks this pigmentation; Lachaise et al. 2000). In the laboratory, male *D. yakuba*/*D. santomea* hybrids are sterile but female hybrids are fertile and can be backcrossed to either parental species (Lachaise et al. 2000; Cariou et al. 2001).

Molecular phylogenetic analysis shows that *D. yakuba* and *D. santomea* are sister species among the nine species of the monophyletic *D. melanogaster* subgroup (Lachaise et al. 2000; Cariou et al. 2001). This pair thus represents a speciation event independent of the well-studied speciation event separating *D. melanogaster* from the ancestor of the *D. simulans* complex, and of the two speciation events in which a *D. simulans*–like ancestor produced two island endemics, *D. sechellia* and *D. mauritiana* (Lachaise et al. 1988). Molecular evidence puts the divergence between *D. yakuba* and *D. santomea* at about 400,000 years ago (Llopart et al. 2002), a divergence time similar to that between *D. simulans* and each of its two sister species (~260,000 to 410,000 years; Kliman et al. 2000). The *D. melanogaster* subgroup thus includes three episodes of speciation following island colonization, all occurring at roughly the same time. However, only *D. santomea* and *D. yakuba* have a well-demarcated insular hybrid zone that allows us to test for reinforcement. Moreover, the two species thus have the prerequisites for testing whether reinforcement has occurred: substantial postzygotic isolation as well as some prezygotic isolation and overlapping ranges probably reflecting secondary contact. Given their biogeography, partial interfertility, and relationship to other species in their group, *D. santomea* and *D. yakuba* are excellent subjects for field and laboratory studies of speciation.

Here we characterize the degree, nature, and genetics of sexual isolation between these species, using a variety of geographic strains that also allow us to also test for the pattern of reinforcement. We also quantify courtship behavior in interspecific mating trials, which implicates the behavioral mechanisms and sex-specificity involved in sexual isolation. Finally, we investigate the degree of sexual isolation of interspecific hybrids, which is relevant to understanding evolutionary processes occurring in the zone of overlap.

Materials and Methods

All flies were reared at 24°C on standard cornmeal-yeast-agar medium, and kept in incubators on a 12-h light/dark cycle.

Strains of both species were derived from flies collected using banana baits. All stocks of *D. yakuba* and *D. santomea* were founded from single females captured in the wild, with the exception of synthetic stocks. The latter, as described below, were formed by combining several isofemale lines from a single geographic population. Such lines were intended to reconstitute the genetic variability of the population and eliminate behaviors due to inbreeding in isofemale lines. We thus have far fewer synthetic strains than isofemale lines. Measurements of sexual isolation and courtship latency and duration were conducted on both isofemale lines and synthetic strains. The collecting locations, dates, and other information about these strains are summarized in Electronic Appendix A, currently available from the *Evolution* Editorial Office at: evolution@asu.edu; below we give the numbers and geographic sources of the flies.

Isofemale Strains

Drosophila santomea isofemale lines

We used 11 isofemale lines of *D. santomea*. Eight of these were derived from females collected in 1998 and 2001 in the zone of overlap, and three derived from females collected in 2001 above the zone of overlap, between 1490 m and 1600 m elevation. The latter were considered parapatric to *D. yakuba* strains collected within the zone.

Drosophila yakuba isofemale lines

We used 22 isofemale lines of *D. yakuba*. Six of these were derived from flies collected in 1998 and 2001 within the zone of overlap, ranging from 1153 m to 1200 m elevation. Five lines were derived from flies collected on São Tomé, but outside the zone of overlap; of these, four were collected in 2000 16 km from the area of overlap and one (collected in 2001) only slightly below the area of overlap (1000 m elevation). The latter line was considered parapatric to *D. santomea* lines collected within the zone of overlap. Two lines were derived from flies collected in 2001 at Anton on Príncipe Island, 175 km northeast of São Tomé (no *D. santomea* have been collected on Príncipe). Nine lines were derived from flies collected on mainland Africa: one from Cameroon (collected in 1967), two from the Täi rainforest on the border between Liberia and the Ivory Coast (collected in 1981), and six from outside Abidjan on the southeastern Ivory Coast (collected in 1999).

Synthetic Strains

Synthetic strains were constructed by combining several isofemale lines, using equal numbers of flies from each line and allowing genic admixture in the stock for at least five generations before flies were used for matings.

Drosophila santomea synthetic strains

The *D. santomea* parapatric (''para'') synthetic strain was a combination of flies from four isofemale collected in 2001 between 1566 m and 1600 m elevation. This strain could be considered parapatric, sympatric, or allopatric to *D. yakuba* depending on the migration distance of flies; it was considered as both sympatric and allopatric to *D. yakuba* in independent tests of reinforcement (see below). The STO synthetic strain was a combination of flies from six isofemale lines collected in 1998 within the zone of overlap. This strain was considered sympatric to *D. yakuba*. The *D. santomea* 2001 synthetic strain was a combination of flies from three

TABLE 1. Degree of sexual isolation for allopatric (A) and sympatric (S) crosses. S/A means that one of the lines is outside the hybrid zone but close to it, that is, parapatric. In some pairings their geographic status was considered either sympatric or allopatric (S/A) because one of the lines was close to the hybrid zone, whereas the other was within the hybrid zone (see text). N is the number of pairings observed for each mating type. In the four mating columns, Y refers to *Drosophila yakuba*, S to *D. santomea*, and the species of the female in each pairing is given first. SI(χ^2) is the statistic of sexual isolation discussed by Gilbert and Starmer (1985), and SI(yak) is the degree of sexual isolation for *D. yakuba* females only (no. Y × S matings/no. Y × Y matings).

Cross (*D. santomea* × *D. yakuba*)	Allopatric (A)/ sympatric (S)	N	Matings				SI(χ^2)	SI(yak)
			Y × Y	Y × S	S × Y	S × S		
Isofemale lines								
STO.5 × Abidjan 60	A	80	45	5	0	22	0.76	0.11
STO.18 × Abidjan 96	A	142	92	23	16	93	0.65	0.25
CAR 1600.1 × Täi18	A	80	58	14	0	52	0.78	0.24
CAR 1490.6 × 115 Cameroon	A	80	58	20	8	51	0.60	0.34
OBAT 1200.14 × Anton-1	A	80	44	7	0	20	0.70	0.16
CAR 1566.3 × Anton-2	A	80	45	9	3	30	0.70	0.20
STO.15 × SJ3	A	80	35	10	10	67	0.60	0.29
STO.4 × SJ2	A	166	55	24	8	94	0.61	0.44
STO.10 × COST 1235.1	S	80	49	3	2	29	0.82	0.06
STO.5 × COST 1235.2	S	80	29	6	2	22	0.72	0.21
OBAT1200.13 × BAR 1000.2	S/A	80	43	3	0	29	0.89	0.07
STO.4 × SA.2	S	80	14	8	1	38	0.56	0.57
STO.15 × SA.3	S	80	53	22	0	52	0.68	0.42
COST 1235.1 × BOSSU 1153.1	S	90	17	5	2	48	0.62	0.29
STO.18 × SA.1	S	80	24	1	7	47	0.72	0.04
CAR 1600.1 × SA.4	S/A	80	32	7	1	50	0.79	0.22
Synthetic lines								
Para synthetic × Täi	A	80	63	21	6	57	0.64	0.33
2001 × Gabon	A	80	43	12	3	44	0.71	0.28
STO × SJ	A	80	45	12	9	57	0.65	0.27
2001 × Abidjan	A	80	65	21	2	48	0.67	0.32
STO × D. Anton Principe	A	80	42	15	7	62	0.63	0.36
STO × 115 Cameroon	A	80	40	16	13	49	0.50	0.40
STO × SA	S	80	77	10	18	46	0.59	0.13
2001 × 2001 sympatric	S	80	28	15	4	46	0.57	0.54
Para synthetic × 2001 sympatric	S/A	80	36	12	4	49	0.67	0.33

isofemale lines collected in 2001 within the zone of overlap. This strain was also considered sympatric to *D. yakuba*.

Drosophila yakuba synthetic strains

The *D. yakuba* Täi synthetic line was a combination of flies from four isofemale lines collected in the Täi rainforest as described above. This strain was considered allopatric to *D. santomea*. The SJ synthetic strain was a combination of four isofemale lines collected in 2000 on São Tomé 16 km from the zone of overlap. This strain was considered allopatric to *D. santomea*. The SA synthetic line was a combination of four lines collected in 1998 within the zone of overlap and was considered sympatric to *D. santomea*. The 2001 sympatric synthetic strain was a combination of four lines collected in 2001 within the zone of overlap and was considered sympatric to *D. santomea*. The Abidjan synthetic strain was a combination of six isofemale lines collected from Abidjan, Ivory Coast (see above). This strain was considered allopatric to *D. santomea*. The Anton-Príncipe synthetic strain was a combination of two isofemale lines from Príncipe Island as described above; this strain was considered allopatric to *D. santomea*. The *D. yakuba* Gabon synthetic strain was constructed by combining three stocks collected in 1998 in Lopé, Gabon. This strain was considered allopatric to *D. santomea*.

Survey of Sexual Isolation

For each pair of strains chosen (one *D. santomea* and one *D. yakuba*), we performed sets of no-choice sexual-isolation tests, each involving pairings between single males and females. Each individual test involved 40 vials divided into four sets of 10: *D. santomea* female with *D. santomea* male (S × S), *D. yakuba* female with *D. santomea* male (Y × S), *D. santomea* female with *D. yakuba* male (S × Y), and *D. yakuba* female with *D. yakuba* male (Y × Y). We made least eight replicates of each test, giving a sample size ranging between 80 and 166 observations of each of the four pairwise combinations (Table 1). Tests were conducted between 0900 h and 1100 h under a constant light and temperature regime (21–23°C), with each observation period lasting 45 min. For each pair we recorded whether copulation occurred and, if so, its latency (the time elapsing between the insertion of flies into vials and the onset of copulation), and, for isofemale lines only, the duration of copulation.

Sexual isolation was measured using the chi-square index of Gilbert and Starmer (1985). When equal numbers of the four types of pairings are observed, this index is calculated as $4(AD - BC)/N^2$, where A and D are the numbers of the two homospecific matings, B and C the numbers of the two heterospecific matings, and N is the total number of observed matings ($A + B + C + D$). This index ranges from -1

(complete disassortative mating) to 1 (complete assortative mating). Gilbert and Starmer's (1985) study shows that, in simulations of multiple-choice mating tests, this index is much less sensitive than other indices of sexual isolation to differences in mating vigor or unequal size of mating pools.

We calculated another index of sexual isolation using *D. yakuba* females, which, unlike our *D. santomea* strains, occur in both sympatry and complete allopatry with the other species. For a given interspecific pairing, this index was simply the number of *D. yakuba* females who mated with *D. santomea* males divided by the number who mated with conspecific *D. yakuba* males. The index ranges from zero (complete sexual isolation of *D. yakuba* females) to one (no sexual isolation of *D. yakuba* females). We calculated this statistic because—as all *D. santomea* lines were collected either in or close to the zone of overlap—we would expect to see more reinforcement in *D. yakuba* than in *D. santomea* females. We also expect to see more reinforcement in females than in males because females, with their substantial investment in eggs, have more to lose by mating with members of the wrong species (Partridge and Parker 1999).

Finally, for comparison to the results of Coyne and Orr (1989, 1997) we calculated their index of sexual isolation, which is 1 − (total number of heterospecific matings/total number of homospecific matings). This index ranges from $-\infty$ (complete disassortative mating) to one (complete sexual isolation).

Because all of our *D. santomea* strains were collected within the zone of overlap or close to it, the tests for reinforcement involved comparing sexual isolation between *D. santomea* strains and *D. yakuba* strains collected either inside or outside the zone of overlap.

Courtship Behaviors

To determine whether sexual isolation was due to a failure of males to court heterospecific females, the rejection by females of courting heterospecific males, or both, we observed individual pairs of flies in a set of matings involving *D. santomea* from the 2001 synthetic strain and *D. yakuba* from the Abidjan synthetic strain. For this pair of strains, all four pairwise combinations (S × S, S × Y, Y × S, and Y × Y) were watched by two observers, each watching one pair at a time and with the pairs randomized among observers so that all four combinations were watched in two sessions per day. Each observation period lasted 30 min and was conducted in an 8-dram, food-containing vial placed on its side. Twenty-four replicate observations were made for each of the four pairs.

The following behaviors were recorded for each pair (Coyne et al. 1994): (1) courtship latency: the time elapsing from when the pairs were placed in a vial until male courtship began (male orienting toward female and extending and vibrating wing); (2) copulation attempts: male orients behind female and curls abdomen underneath, unsuccessfully attempting genital contact; (3) copulation latency: the time elapsing between the introduction of pairs into the vial and the onset of copulation; and (4) proportion of time spent courting; when pairs did not copulate, this statistic was calculated as the proportion of time between the first time of courtship (courtship latency) and the end of total 30-min observation period during which the male courted the female. When copulation occurred, this statistic was calculated as the proportion of time between the first courtship and copulation during which the male courted the female. We defined courtship as occurring when the male followed the female and performed wing displays. We also recorded the number of matings occurring in each combination of male and female.

Sexual Isolation and Mating Behavior of Interspecific Hybrids

In this experiment we observed the behavior of the two genotypes of F_1 hybrid males (from reciprocal hybridizations) to that of pure-species males when all three types of males were tested with pure-species females. We also observed the behavior of F_1 hybrid females compared to pure-species females when presented with males of the two pure species. These crosses give an idea of the dominance of genes affecting sexual isolation of both species. For these tests we used two allopatric strains that showed substantial sexual isolation: *D. yakuba* Abidjan 96 and *D. santomea* STO.18.

F_1 hybrid males were generated by crossing the two strains in both directions, producing hybrids having either a *D. santomea* or *D. yakuba* X chromosome. In one set of observations, these two types of males—as well as pure *D. yakuba* and pure *D. santomea* males—were placed individually in vials with a single *D. santomea* female, so that four pairs of flies were watched together. In another set of observations, the same four types of males were tested against pure *D. yakuba* females. All observations lasted for 30 min, and we recorded presence or absence of copulation, copulation latency, and copulation duration. A total of 120 observations were made of each of the four mating combinations (12 replicates with 10 pairs of each mating type per replicate).

Hybrid females were produced in only one reciprocal cross (*D. yakuba* Abidjan 96 females × *D. santomea* STO.18 males). These F_1 females were tested along with both pure-species females in two sets of experiments. The first involved placing the three types of females with pure *D. santomea* males; the second with pure *D. yakuba* males. As described above, mating observations lasted 30 min and involved 12 replicates, each of which contained 10 pairs of the three mating combinations.

RESULTS

Survey of Sexual Isolation

Magnitude of sexual isolation

Twenty-five pairs of strains were used in this survey of sexual isolation, 16 involving isofemale lines and nine involving synthetic strains. Some of the *D. santomea* isofemale lines were used twice, but in such cases one pairing was with an allopatric strain of *D. yakuba* and the other with a sympatric strain of *D. yakuba*. Some of the synthetic lines of both species were used in more than one test of sexual isolation; this was unavoidable given the limited number of strains available. It is possible that using a strain more than once makes the data nonindependent and inflates the degrees of freedom. However, because we are assessing sexual isolation

(and, in other tests, copulation duration and latency), one can make a case that each pairing between strains is independent. At any rate, the main results described below—that sexual isolation is strong and asymmetric and that there is no evidence for reinforcement—are so clear-cut that the possibility of nonindependence seems unimportant.

Table 1 gives the numbers of matings observed and two indices of sexual isolation (omitting the index of Coyne and Orr 1989) for all 25 mating tests, classified by whether the pairs were allopatric (A), sympatric (S), or parapatric (S/A), and whether the tests used isofemale lines or synthetic strains. Several conclusions are obvious.

First, the species show strong sexual isolation in the laboratory. The index of Gilbert and Starmer (1985) gives a mean sexual isolation of 0.735 ± 0.018 (SE), among all 25 pairs of strains, with values ranging from 0.50 to 0.89. (The theoretical maximum of 1.0 is attained only when the two types of conspecific matings occur with equal frequency and there are no heterospecific matings.) No pairs of strains were weakly isolated. Given that these are no-choice tests conducted in the laboratory, it is not likely that sexual isolation occurring in nature is substantially weaker than that tested here, and may well be stronger. The average *D. yakuba* index of sexual isolation (the number of heterospecific matings involving the *D. yakuba* female divided by the number of conspecific *D. yakuba* matings) was more variable, ranging from 0.07 to 0.57, with a mean of 0.275 ± 0.028. (For this index, the greatest possible sexual isolation gives a value of zero and the lowest gives one.) The mean index of sexual isolation devised by Coyne and Orr (1989) is 0.827 ± 0.014, close to the maximum value of 1.0.

Second, the sexual isolation is asymmetrical: matings between *D. yakuba* females and *D. santomea* males are usually more common than matings in the opposite direction. This was the case in 22 of the 25 pairings (Table 1). The mean number of matings in each test between *D. santomea* females and *D. yakuba* males was 5.04 ± 1.02 and between *D. yakuba* females and *D. santomea* males was 12.04 ± 1.37. A paired *t*-test between the numbers of these reciprocal matings across all 25 pairings shows a highly significant difference (t_{24} = 5.017, $P < 0.0001$). This pattern of asymmetrical sexual isolation is common in *Drosophila* and has been seen in other animals as well (see Coyne and Orr 1998).

In contrast, there is no difference in mating propensity for the two conspecific matings in our test: the mean number of S × S matings (48.08 ± 3.7) does not differ systematically from the mean number of Y × Y matings (45.28 ± 3.56): among the 15 hybridizations, paired $t_{24} = 2.80$, $P = 0.4816$.

Geographic pattern of sexual isolation

We found no evidence for reinforcement in these hybridizations, because there is no difference in the degree of sexual isolation between sympatric and allopatric pairs of lines or strains. This result is independent of the measure of sexual isolation that we use, or whether we classify the parapatric strains as being involved in sympatric or allopatric hybridizations. For example, if all the S/A pairs in Table 1 (those involving one line or strain collected close to the zone of overlap) are counted as sympatric, the mean sexual isolation using the chi-square measure is 0.745 ± 0.030 for the 11 sympatric pairs and 0.728 ± 0.023 for the 14 allopatric pairs. This is not significant using either the nonparametric Mann-Whitney *U*-test ($Z = 0.52$, $P = 0.60$), or an unpaired *t*-test ($t_{23} = 0.41$, $P = 0.69$). If the three S/A pairs are all counted as allopatric, the mean sexual isolation using the chi-square measure is 0.709 ± 0.087 for the eight sympatric pairs and 0.748 ± 0.091 for the 17 allopatric pairs. This difference is also not significant and is, in fact, in the direction opposite to that predicted by reinforcement. As might be expected from these results, none of the eight possible permutations of S/A pairs into either A or S classes gives a significant difference in sexual isolation between sympatric and allopatric strains.

The sexual isolation index for *D. yakuba* females might be more sensitive to the existence of reinforcement than is the chi-square index, for *D. santomea* females, being collected near or within the zone of overlap, might not show a difference in discrimination when tested with sympatric or allopatric *D. yakuba* males. Nevertheless, this index of sexual isolation also shows no difference between allopatric and sympatric pairings under all permutations of the S/A designations. If, for example, all S/A populations are counted as sympatric, the mean sexual isolation for the 11 sympatric pairings is 0.262 ± 0.056 and for the 14 allopatric pairings is 0.285 ± 0.024. These differences are not significant using either the unpaired *t*-test ($t_{23} = 0.41$, $P = 0.69$) or the Mann-Whitney *U*-test ($Z = 0.63$, $P = 0.53$).

Thus, using either statistic, we find no hint of reinforcement in these species. Given our sample sizes, we certainly would have detected the substantial degree of reinforcement seen in other studies of *Drosophila* species with overlapping ranges (Ehrman 1965; Wasserman and Koepfer 1977; Noor 1995).

Copulation latency and duration

Electronic Appendix B (currently available from the *Evolution* Editorial Office at: evolution@asu.edu) gives copulation latencies and durations for all four types of matings in the 25 comparisons between strains. Comparing the pure-species matings (Y × Y and S × S), one finds a marginally significant difference between copulation latencies, with that of *D. yakuba* being slightly higher than that of *D. santomea* (unpaired $t_{36} = 2.05$, $P = 0.04$). While the copulation latencies of the two hybrid matings do not differ from one another (paired $t_{19} = 1.30$, $P = 0.21$), the mean courtship latency for conspecific pairings (19.98 ± 0.59 min, $n = 38$; weighted means are used for strains involved in more than one pairing) is significantly lower than for all combined heterospecific matings (25.10 ± 0.92 min, $n = 45$; comparison using unpaired $t_{81} = 4.48$, $P < 0.0001$; using Mann-Whitney *U*: $Z = 4.34$, $P < 0.0001$). The higher copulation latency of heterospecific matings may reflect a greater reluctance of females to mate heterospecifically than indicated by the index of sexual isolation. The elevated latency of heterospecific matings may also contribute to reproductive isolation in nature because heterospecific courtships, lasting longer, are more likely to be interrupted.

The duration of copulation for all classes of matings was

TABLE 2. Courtship behavior statistics for the pairings involving the *Drosophila santomea* (S) 2001 synthetic strain and the *D. yakuba* (Y) Abidjan synthetic strain. In each pairing, the species of the female is listed first. Twenty-four trials were made for each of the four pairings. Standard errors for all statistics are given in parentheses.

	Mating			
	S × S	S × Y	Y × S	Y × Y
No. of matings	19	1	8	23
Courtship latency (min)	2.63 (0.78)	3.18 (0.41)	3.06 (0.52)	2.84 (0.79)
Copulation attempts	0.52 (0.24)	1.33 (0.94)	0.35 (0.15)	0.13 (0.07)
Copulation latency (min)	10.61 (1.36)	22.55	10.60 (2.27)	7.70 (1.32)
Proportion of time spent courting	0.29 (0.054)	0.11 (0.040)	0.18 (0.50)	0.47 (0.053)

roughly similar, lasting about 26 min except for Y × Y copulations, with a mean of 37.3 min (Table 2). An analysis of variance for copulation duration across all four types of matings showed significant heterogeneity ($F_{3,50} = 8.49$, $P = 0.0001$). This heterogeneity was due entirely to the greater length of conspecific *D. yakuba* copulations. Of the six unplanned pairwise comparisons between mating types using Fisher's protected least significant difference test (PLSD; Sokal and Rohlf 1995), the only significant differences were seen in comparisons involving the Y × Y mating (all comparisons were significant at $P = 0.0016$ or lower). Among the 16 isofemale pairings, 14 showed longer Y × Y than S × S matings, and an unpaired *t*-test using weighted means of both species confirmed the significance of this difference ($t_{25} = 4.06$, $P = 0.004$). There has thus been significant evolutionary change in the duration of intraspecific copulation during the divergence between *D. yakuba* and *D. santomea*. Given the lack of information about copulation duration in the nearest outgroups, we cannot draw any conclusions about the direction of evolution.

Courtship Behaviors

Table 2 gives the data on courtship behavior and copulation latency for 24 observations of the four possible pairings between flies from the *D. yakuba* Abidjan synthetic strain and the *D. santomea* 2001 synthetic strain (see also Table 1 and Electronic Appendix B for statistics on sexual isolation, copulation latency, and copulation duration within and among these strains). The sexual isolation between this pair of strains is high: Gilbert and Starmer's (1985) index is 0.66, nearly identical to the 0.67 value calculated for these strains in the independent test shown in Table 1. As with most heterospecific crosses involving these species, there are more matings between *D. yakuba* females and *D. santomea* males than in the reciprocal pairing.

Analyses of variance for three parameters of courtship (excluding the arcsine-transformed values of proportion of time courting) show no significant heterogeneity among these four pairings. Because much of these data have large standard errors (particularly copulation attempts), a larger sample would be needed to detect smaller differences. There is, however, no obvious indication that males involved in heterospecific pairings attempt copulation less frequently than do males involved in homospecific pairings.

Table 2 shows that, compared to conspecific pairings, heterospecific pairings showed a significantly smaller percentage of time during which males courted females. The overall ANOVA for arcsine-transformed values of proportion of time courting showed a significant heterogeneity among pairings ($F_{3,90} = 0.65$, $P < 0.0001$). Post hoc PLSD tests showed that this heterogeneity was due largely to the courtship times in heterospecific matings. Pairwise comparisons showed significant differences between the S × S and S × Y pairings ($P = 0.02$), the Y × Y and S × Y pairings ($P < 0.0001$), the Y × Y and Y × S pairings ($P = 0.0001$) and the two homospecific pairings (S × S vs. Y × Y; $P = 0.013$). This result, combined with casual observations of other strains showing females repeatedly rejecting the courtship of heterospecific males, indicates that sexual isolation between these strains involves not only a reduced courtship intensity of males when confronted with heterospecific females, but also a reduced female preference for heterospecific males. However, the asymmetry of sexual isolation (i.e., the greater frequency of heterospecific pairings involving *D. yakuba* than *D. santomea* males) appears to reflect only a difference in female preference: the S × Y and Y × S pairings did not differ significantly in any measure of male courtship intensity.

Sexual Isolation, Copulation Duration, and Copulation Latency of Interspecific Hybrids

The analysis of sexual behavior of hybrids involved testing F_1 hybrids of either sex against pure-species individuals of the opposite sex. Hybrids were not tested against other hybrids. This analysis was designed to determine the direction of dominance, if any, for sexual isolation of F_1 hybrids, information that is required for further genetic analysis through backcrossing. This test also gives information about whether the source of the X chromosome in males has a significant effect on sexual isolation and whether hybrid females show sexual isolation against pure-species males. Finally, this experiment also provides information on the degree of dominance in hybrids for any interspecific differences in courtship latency and duration. Table 3 gives the data for the four sets of observations.

Sexual isolation

In the first two sets of studies shown in Table 3 (sections A and B), males of all four types (pure *D. yakuba*, pure *D. santomea*, F_1 males with *D. santomea* mother, and F_1 males with *D. santomea* father) were tested against either *D. santomea* females or *D. yakuba* females. In both studies, the sexual isolation between species is evident from comparing

TABLE 3. Copulation duration and latency of hybrid matings. Table gives the number of copulations occurring (matings) of 120 trials for each combination of males and females, as well as the latency and duration of copulations that did occur. S, *Drosophila santomea* STO.18 line; Y, *D. yakuba* Abdijan 96 line. For each mating, the female parent is given first. Hybrid genotypes listed as XY (male) or XX (female), with the source of the X or Y given in subscripts as y (*D. yakuba*) or s (*D. santomea*).

Tests involving hybrid males

A. F_1 males × STO.18 females

	Mating type			
	S × S	S × X_sY_y	S × X_yY_s	S × Y
No. matings	68	50	80	8
Copulation latency (min)	18.48 (1.23)	19.97 (1.52)	17.31 (1.09)	27.21 (2.34)
Copulation duration (min)	30.41 (0.98)	33.39 (1.08)	36.41 (1.03)	36.13 (6.05)

B. F_1 males × *D. yakuba* 96 females

	Y × Y	Y × X_sY_y	Y × X_yY_s	Y × S
No. matings	83	43	85	12
Copulation latency (min)	18.17 (1.19)	22.44 (1.55)	15.48 (0.95)	25.51 (3.42)
Copulation duration (min)	58.69 (1.02)	38.55 (1.58)	44.99 (0.92)	31.56 (1.75)

Tests involving hybrid females

C. F_1 females × STO.18 males

	Mating type		
	S × S	X_sX_y × S	Y × S
No. matings	70	79	18
Copulation latency (min)	19.11 (1.18)	17.68 (1.18)	26.53 (2.25)
Copulation duration (min)	28.60 (1.06)	31.59 (0.96)	28.92 (1.99)

D. F_1 females × *D. yakuba* 96 males

	Y × Y	X_sX_y × Y	S × Y
No. matings	84	85	10
Copulation latency (min)	16.46 (0.96)	14.47 (1.15)	22.14 (4.13)
Copulation duration (min)	61.78 (1.33)	57.67 (1.50)	41.99 (5.67)

the number of homospecific and heterospecific matings. Moreover, F_1 hybrids with the *D. santomea* X chromosome (X_sY_y) mate significantly less often than do hybrids with the *D. yakuba* X chromosome (X_yY_s), regardless of whether they are tested against *D. santomea* females ($\chi^2_1 = 6.92$, $P < 0.001$) or *D. yakuba* females ($\chi^2_1 = 13.78$, $P < 0.001$). However, X_yY_s males mate just as frequently with females of either species as do their conspecific males.

Unless the difference between the reciprocal F_1 males is due to a difference in cytoplasm or Y-chromosomal loci, the results given above show a significant effect of the X chromosome on sexual isolation. The nature of the effect, however, is surprising. If the X chromosome carried genes involved in sexual isolation of males, we would normally expect X_yY_s males to mate more readily with *D. yakuba* females than would X_sY_y males (which is the case), but also that this difference would be reversed with *D. santomea* females (which is not the case).

The observations that the X_yY_s hybrid male is always less isolated than the X_sY_y male, regardless of the female's species, and such males mate with females of either species as readily as do the females' conspecific males, suggest two conclusions. First, the *D. santomea* X chromosome may carry genes that, in combination with genes on *D. yakuba* autosomes, cause male behavioral sterility, so that X_sY_y males either court all females less vigorously than do X_yY_s males or have some debility that makes them unacceptable to females (for similar examples, see Stratton and Uetz 1986; Noor 1997; Noor et al. 2001). Such behavioral sterility may mask any species-specific effects of the X chromosome on sexual isolation of males. However, the observation that X_yY_s males mate as frequently as do *D. santomea* males when tested with *D. santomea* females, and as frequently as do *D. yakuba* males when tested with *D. yakuba* females, suggests that the X chromosome does not in fact carry genes with a large effect on sexual isolation of males. Moreover, the autosomal alleles in males that cause sexual isolation act recessively against females of both species: X_yY_s hybrid males are heterozygous at *all* autosomal loci, but mate as readily with any pure-species female as do her conspecific males.

Similar recessivity for sexual isolation is seen when one tests hybrid females against males of both species (Table 3C, D). When tested with *D. santomea* males, the X_sX_y F_1 females mate as frequently as do pure *D. santomea* females and, when tested with *D. yakuba* males, mate as frequently as do pure *D. yakuba* females (chi-square values testing 70 vs. 79 and 84 vs. 85 matings are not significant). One conspecific copy of each autosomal and X-linked gene in females renders them as attractive to (and as liable to mate with) any male as are his conspecific females.

Copulation latency and duration

As noted above, sexual isolation is partly evinced as a difference in copulation latency between pure-species matings and heterospecific matings. For both experiments using hybrid males (Table 3A, B), copulation latency is significantly heterogeneous among all four pairings. Using *D. san-*

tomea females (Table 3A), one finds $F_{3,202} = 2.72$, $P = 0.045$, with the heterogeneity being due, according to Fisher's PLSD test, to a difference between the latency of pure *D. yakuba* males and either pure *D. santomea* males ($P = 0.02$) or X_yY_s F_1 males ($P = 0.008$). In the test for copulation latency with *D. yakuba* females (Table 3B), one finds $F_{3,219} = 6.78$, $P = 0.0002$, with both pure *D. yakuba* males and X_yY_s F_1 males having significantly shorter latencies than do *D. santomea* males (PLSD, $P = 0.018$ and 0.0013, respectively). The X_sY_y F_1 males also have significantly longer copulation latency than do either pure *D. yakuba* males (PLSD, $P = 0.024$) or X_yX_s males ($P = 0.0003$). In Table 3A and B, X_sY_y heterospecific males have longer copulation latency than conspecific males, perhaps again reflecting behavioral sterility of hybrid males with a *D. santomea* X chromosome.

Although the higher copulation latency of heterospecific than conspecific flies is also seen in the tests using hybrid females (e.g., compare Y × S with S × S matings in Table 3C), hybrid females do not show reduced acceptance by pure-species males. In tests involving males of both species (Table 3C, D), hybrid females have lower copulation latencies than do pure conspecific females.

As in the tests on isofemale lines, copulation duration is longer for conspecific *D. yakuba* matings than for conspecific *D. santomea* matings. This appears to be a species-specific difference. The Abidjan 96 *D. yakuba* copulation is extraordinarily long (about 60 min), whereas the *D. santomea* STO.18 copulation lasts about 30 min (Table 3B, D), well within the range of other strains of this species (Table 2). The species difference appears to depend on both sexes. In Table 3B, for example, the copulation involving *D. yakuba* females is significantly shorter when it involves *D. santomea* males (Y × S matings) than conspecific *D. yakuba* males (Y × Y matings; PLSD, $P = 0.018$), showing that males play a role in the interspecific difference in copulation duration. The involvement of females is seen by the considerably shorter duration of S × Y than Y × Y matings (Table 3D, PLSD $P < 0.0001$; see also comparison of S × Y matings in Table 3A with Y × Y matings in Table 3B).

There is a suggestion that in males, the X chromosome might contribute to this difference, as X_yY_s males mate longer than X_sY_y males whether tested against either *D. santomea* or *D. yakuba* females (Table 3A, B). The difference in copulation duration between these hybrids is significant when both are tested against *D. yakuba* females (Table 3B; PLSD $P = 0.0003$), but of borderline significance when tested against *D. santomea* females (Table 3A; PLSD, $P = 0.0533$). Using Fisher's test for combining probabilities from independent tests of significance, we find $\chi^2_4 = 22.09$, $P < 0.001$, indicating an overall significant effect of the X chromosome on copulation duration.

We are unable to test the effects of the X chromosome in females, but hybrid females appear to have copulation durations similar to but slightly longer than those of pure *D. santomea* females when tested against *D. santomea* males (Table 3C: PLSD of hybrid females versus *D. santomea* males, $P = 0.05$). Likewise, hybrid females have copulation durations slightly shorter than those of pure *D. yakuba* females when tested against *D. yakuba* males (Table 3D, PLSD $P = 0.05$), but significantly longer than those of pure *D. santomea* females when tested against pure *D. yakuba* males (Table 3D, PLSD $P = 0.0006$). Thus, when tested against *D. yakuba* males, the genes in *D. yakuba* females that cause longer copulation are largely dominant.

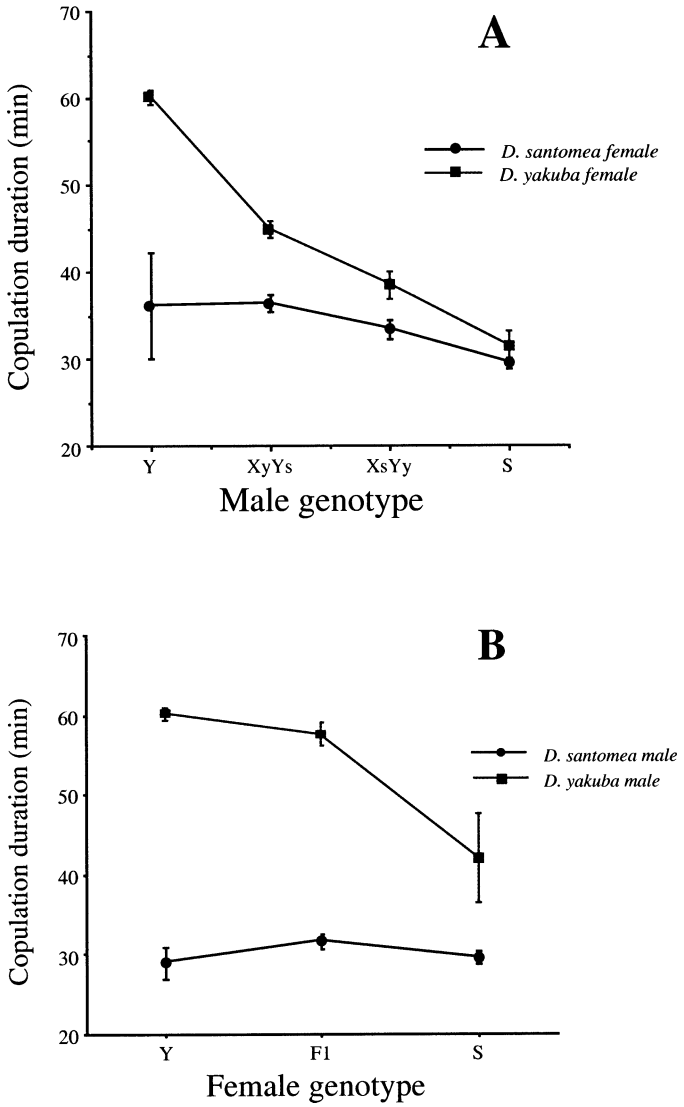

FIG. 1. (A) Copulation duration of pure-species and hybrid males (from the two reciprocal crosses) when mated with pure-species females. Data are taken from Table 3, with the durations of some matings (i.e., *Drosophila santomea* females × *D. yakuba* males) taken as the unweighted average of values from two of the sets of crosses given in Table 3. Error bars represent one standard error (when two identical matings are combined from separate experiments, these estimates are for the combined data). (B) Copulation duration of pure-species and hybrid females (the latter derived from the cross between *D. yakuba* females and *D. santomea* males) when mated with pure-species females. Data are taken from Table 3, with the durations of some matings (i.e., *D. santomea* females × *D. yakuba* males) taken as the unweighted average of values from two of the sets of crosses given in Table 3. Error bars represent one standard error (when two identical matings are combined from separate experiments, these estimates are for the combined data).

These results are grasped more easily from graphs. Figure 1A and 1B show, respectively, the copulation times for males tested against pure-species females and for female tested

against pure-species males. Figure 1A demonstrates the effect of the X chromosome in males on copulation duration, which is more pronounced in hybridizations involving *D. yakuba* than *D. santomea* females. This figure also shows the effect of the autosomes in males (compare the copulation duration of *D. yakuba* males with that of F_1 X_yY_s males when both are tested against *D. yakuba* females, and the copulation duration of *D. santomea* males with that of F_1 X_sY_y males when both are tested against either *D. santomea* or *D. yakuba* females). In males, then, a minimum of two loci (at least one on the X chromosome and one on the autosomes) are responsible for the species difference in copulation duration.

Figure 1B shows that female genotype has little effect on copulation duration when tested against *D. santomea* males, but a large effect when tested against *D. yakuba* males. The plot for *D. yakuba* males in Figure 1B also shows the dominance of the genes in *D. yakuba* females that cause longer copulation duration.

Discussion

Sexual isolation between *D. yakuba* and *D. santomea* is strong. Coyne and Orr's (1989) isolation index gives a value of 0.827, not far from the maximum value of 1.0. This degree of isolation is typical of recently diverged species that are at least partially sympatric, but is almost never observed between allopatric taxa of similar age (Coyne and Orr 1997). Coyne and Orr (1989, 1997) suggest that the pattern of higher sexual isolation in sympatric than in allopatric taxa probably involves reinforcement. However, reinforcement is unlikely to explain the strong sexual isolation between *D. santomea* and *D. yakuba*, because we found no pattern of reinforcement in our survey of geographic strains.

Sexual isolation is also asymmetrical, with one interspecific cross proceeding much more readily than the reciprocal cross. This accords with observations in many species of *Drosophila* (e.g., Watanabe and Kawanishi 1979; Kaneshiro and Giddings 1987; Robertson 1988) and other species as well (Arnold et al. 1996). This asymmetry has received some attention, but its causes are not well understood. Arnold et al. (1996) proposed that mating asymmetry can arise as a transitory phenomenon under the run-away model of sexual selection occurring in isolated populations. Such asymmetry, however, is often seen even among distantly related species of *Drosophila*, in which sexual isolation is nearly complete in one direction (Coyne and Orr 1989). The asymmetry of sexual isolation remains an important but unresolved evolutionary problem.

Our mating tests give no evidence for reinforcement in *D. santomea* and *D. yakuba*, despite the fact that these species seem to have all the biogeographic and evolutionary prerequisites for the evolution of reinforcement. The species probably began diverging in allopatry, with *D. santomea* evolving after colonization of São Tomé by its mainland ancestor with *D. yakuba*. During the period of allopatry, it is likely that both sexual and postzygotic isolation evolved between the species. It seems reasonable to suppose that the present overlapping distributions of the two species on São Tomé reflect either a secondary invasion of the island by *D. yakuba* or a range expansion of *D. yakuba* if speciation occurred allopatrically on the island. Secondary contact is, of course, a prerequisite for reinforcement.

In addition, recent theories (Liou and Price 1994; Kelly and Noor 1996) show that high levels of postzygotic and sexual isolation between allopatric populations—as seen in these species—facilitate the evolution of higher sexual isolation in sympatry. Moreover, on São Tomé the species form a zone of overlap, so that on the island they probably conform more to Servedio and Kirkpatrick's (1997) model of symmetrical migration between two demes than to their model of one-way migration between demes. The symmetrical model facilitates the evolution of reinforcement. Finally, the species are known to hybridize in nature, a prerequisite for reinforcement.

There are of course many reasons why reinforcement may not have evolved between *D. santomea* and *D. yakuba*. These include (1) the possibility that contact between the species is quite recent, so that sexual isolation has not yet had time to evolve; the lowland environment of São Tomé has changed dramatically in the last five centuries, with the recent introduction of many lowland plants, and it is possible that *D. yakuba* has been present on the island for only a few hundred years; (2) the absence of genetic variation for increased mate discrimination; (3) the possibility that gene flow between the species is strong enough to override the evolution of increased sexual isolation; and (4) the possibility that gene flow into the zone of overlap is not symmetrical, but much stronger from one species than from the other. Although we have no information about this last possibility, such unbalanced gene flow reduces the probability of reinforcement (Servedio and Kirkpatrick 1997).

However, we feel that the most likely explanation for the absence of reinforcement is premating isolation between the species (which includes but is not limited to sexual isolation) may have been nearly complete by the time their ranges met. The extreme rarity of hybrids in nature (estimated at 1%) may mean that selection for reinforcement—whose strength is proportional to the rate of hybridization—may be very weak. The dearth of observed hybrids, much lower than expected from our laboratory estimates of sexual isolation, can be caused by several factors, including the possibility that sexual isolation in nature is higher than that measured in the laboratory; that there is conspecific sperm precedence, so that females carrying sperm from males of both species will produce mostly conspecific progeny (Price 1997); or that the species show some ecological isolation, preferring different microhabitats and/or resources in the area of overlap. (It is also possible that hybrids are more frequent than 1% but have gone undetected because some F_1 hybrids and nearly all later generation hybrids are morphologically indistinguishable from pure species.)

It is theoretically possible that reinforcement may indeed have evolved on the island, but that its signature (higher sexual isolation in sympatry) may have been lost due to migration of genes for higher mate discrimination out of the zone of overlap, so that either or both species become fixed for genes producing strong sexual isolation (Walker 1974). If, for example, there were strong selection on sympatric *D. santomea* females to discriminate against heterospecific males, but much weaker selection in allopatry, gene flow from

the zone of overlap could homogenize *D. santomea* for the increased degree of preference. However, this scenario is much less likely for *D. yakuba* because it would require repeated invasion of the African mainland by *D. yakuba* from São Tomé. And, of course, we find no evidence for reinforcement in *D. yakuba*.

The study of Cariou et al. (2001) lends some support to our findings of no pattern of higher mate discrimination in sympatry. They observed that interspecific crosses were equally ''successful'' (i.e., produced progeny), regardless of whether the *D. yakuba* parent was from a population sympatric or allopatric with *D. santomea*. These results, not intended as a test of reinforcement, are weaker than ours because sexual isolation can be broken down over a long period of time when heterospecific individuals are confined for several days in vials.

As noted above, Coyne and Orr (1989, 1997) showed that recently diverged pairs of *Drosophila* species that are at least partially sympatric had significantly higher levels of sexual isolation than did allopatric pairs of comparable age. This pattern was ascribed to reinforcement. However, there is an alternative explanation: those species that attained high levels of sexual isolation during an initial phase of allopatry are more likely to remain distinct in sympatry than those that evolved lower sexual isolation, which would cause them to fuse. For several reasons, Coyne and Orr (1989) regarded this differential fusion hypothesis as less likely than reinforcement to explain high sexual isolation in sympatry. Nevertheless, differential fusion may contribute at least partially to the pattern of high sexual isolation among sympatric species and may have done so in *D. yakuba* and *D. santomea*. Obviously, distinguishing reinforcement from other factors that can cause high sexual isolation between sympatric taxa (e.g., differential fusion or character displacement of mating signals; Noor 1999) will require more studies like the one described here.

Beyond sexual isolation and hybrid male sterility, the increased courtship latency of hybrid as opposed to pure-species crosses (an average difference of 5 min) may constitute an additional barrier to gene flow in nature. Courtships in nature, which in this group typically occur at the oviposition site, can be interrupted by either physical factors or male-male competition, and the extended courtship involved in interspecific matings makes them more susceptible to premature termination.

Along with pigmentation, the duration of intraspecific copulation has also diverged during the evolution of *D. santomea* and *D. yakuba*, with the latter having copulations that last about 10 min longer. Without information on copulation duration of the outgroup species (*D. teissieri*; Lachaise et al. 2000), we cannot determine whether long or short duration is the derived condition. It is worth noting, however, that one case of speciation via island colonization in the *D. melanogaster* subgroup (the production of *D. mauritiana* from a colonization by its mainland ancestor with the pan-African *D. simulans*) has produced a similar divergence in copulation duration, with the island species showing shortened copulation as a derived trait (Coyne 1993).

Genetic analysis of F_1 hybrids between *D. santomea* and *D. yakuba* shows that the difference in copulation duration is due to evolutionary changes in both sexes, with at least two genes involved in males (at least one X-linked and one autosomal) and at least one gene in females. These results differ somewhat from similar studies in *D. mauritiana* and *D. simulans*, in which copulation duration is determined largely by the male's genotype, with female genotype having little effect (Coyne 1993).

The genetic analysis of sexual isolation given in Table 3 shows two interesting features. First, sexual isolation in females—their discrimination against heterospecific males—is recessive in hybrids in both directions. That is, F_1 hybrid females will mate as readily with *D. santomea* males as do *D. santomea* females and as readily with *D. yakuba* males as do *D. yakuba* females. This has been seen in similar studies of other *Drosophila* species, especially those in which males appear to court heterospecific females as ardently as they do conspecific females (Noor 2000). The recessivity of preference in hybrids is also a prediction of Noor's (2000) theory that, if altered female mating preferences are themselves deleterious, they can nevertheless spread as pleiotropic by-products of adaptive evolution if the preferences are recessive. Because female mate discrimination is recessive in both directions, Noor's theory would require that female preference in both *D. yakuba* and *D. santomea* have diverged from the mating preference of the ancestral females.

The second notable result of our genetic analysis is that F_1 hybrid males having their X chromosomes from *D. santomea* do not mate as well as do males with the *D. yakuba* X chromosome, regardless of whether they are presented with *D. santomea* or *D. yakuba* females. This may involve behavioral sterility of the X_sY_y males: Their courtship may somehow be deficient compared to X_yY_s males, or these hybrid males have a trait that makes them unattractive to females of either species. A similar instance of behavioral sterility was described in *D. persimilis*/*D. pseudoobscura* hybrids by Noor (1997; see also Noor et al. 2001). In this case, male F_1 hybrids with a *D. persimilis* X chromosome had low courtship intensity, so that they mated with *D. persimilis* females about as well as did F_1 hybrid males having the *D. pseudoobscura* X chromosome. However, the $X_{pseu}Y_{per}$ males mated much more frequently than did the sickly $X_{per}Y_{pseu}$ males when both were presented with *D. pseudoobscura* females.

The studies reported here suggest that *D. santomea* and *D. yakuba* will provide fertile subjects for genetic analysis of reproductive isolation in the laboratory and for fieldwork on the evolutionary dynamics of the hybrid zone.

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