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GENETICS OF SEXUAL ISOLATION AND COURTSHIP DYSFUNCTION IN MALE HYBRIDS OF *DROSOPHILA PSEUDOOBSCURA* AND *DROSOPHILA PERSIMILIS*

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Abstract.—Despite the importance of sexual isolation to speciation, few studies have analyzed the genetic basis of interspecific mating discrimination, particularly using hybrid males. In this study, I investigated the genetic basis of sexual isolation using male hybrids of *Drosophila pseudoobscura* and *D. persimilis*. Hybrid male mating success was caused by interactions between the X-chromosome and autosomes (or Y-chromosome), and different arms of the X-chromosome contributed to mating success with females of each species. Further, although there was an X-chromosome component to mating success, its magnitude was not disproportionately large when compared with the proportion of the genome contained on this chromosome. Some hybrid males courted with an anomalously low intensity, so I simultaneously mapped the genetic basis of this "courtship dysfunction." The courtship dysfunction was caused by an interaction between the left arm of the X-chromosome in *D. persimilis* with the autosomes or Y-chromosome from *D. pseudoobscura*. Anomalous courtship behavior in interspecific hybrids can obscure the conclusions of studies of the genetics of sexual isolation, so courtship intensity should be evaluated in all such investigations.

Key words.—Courtship intensity, *Drosophila persimilis*, *Drosophila pseudoobscura*, mating discrimination, reproductive isolation, speciation.

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"Ethological barriers to random mating constitute the largest and most important class of isolating mechanisms in animals" (Mayr 1963).

In animals, sexual isolation appears to be the most important and widespread form of reproductive isolation (Mayr 1963). Despite its importance, surprisingly few studies have attempted to determine the genetic basis of sexual isolation of sibling-species pairs. Direct genetic analyses of sexual isolation can only be performed in species with mapped morphological, biochemical, or DNA markers (Coyne 1992a), so species of the genus *Drosophila* are particularly well-suited for such studies.

Several questions may be addressed in genetic studies of sexual isolation in *Drosophila*: (1) Are there patterns to the genetic basis of sexual isolation, such as a disproportionately large X-chromosome effect (Ewing 1969; Charlesworth et al. 1987; Ritchie and Phillips, in press)? (2) Do genes that confer sexual isolation act epistatically? (3) Do related species use alternative alleles at the same loci to recognize conspecifics? and (4) What phenotypic characters are involved in the sexual isolation of *Drosophila* species?

Existing studies of *Drosophila* sexual isolation have left some of these questions largely unexplored for two reasons. First, surprisingly few studies have investigated the genetic basis of sexual isolation using hybrid males, and only three studies have directly evaluated the contributions of individual chromosomes to male sexual isolation (Ehrman 1961; Zouros 1981; Coyne 1996). This deficiency hinders our ability to compare the genetic bases of male and female sexual isolation and can hinder our inferences about the evolutionary origin of sexual isolation. For example, Charlesworth et al. (1987) postulated that advantageous recessive or underdominant alleles would accumulate on the sex chromosomes if new alleles were frequently fixed by natural selection. Such an accumulation would not occur in female-specific characters since their X-chromosomes are not hemizygous (Coyne 1992b). Hence, application of the Charlesworth et al. (1987) model to mating discrimination should focus on studies of the genetics of sexual isolation in hybrid males rather than hybrid females.

Second, genetic studies of sexual isolation have been performed in species which may possess discrimination by both males and females. Thus, in behavioral studies of backcross males, species discrimination by males may be confounded with male characters producing sexual isolation from females. These two characteristics might have different genetic bases, and they should be investigated individually. Thus far, only the concurrent study by Coyne (1996) appears to circumvent this problem.

Drosophila pseudoobscura and *D. persimilis* are excellent subjects for a genetic study of male sexual isolation for several reasons. First, hybrid females are fertile, allowing genetic analysis. Second, males court heterospecific females as quickly and intensely as they do conspecific females (Noor 1996a), so genetic studies will not confound male discrimination with male phenotypic characters that females identify. Third, visible genetic mutant stocks are available in both species. Finally, Tan (1946) did a preliminary study of the genetics of female discrimination in these species, so it can be compared with male sexual isolation.

While studying sexual isolation in these species, I noted that some hybrid males courted with abnormally low intensity relative to pure-species males. While overall locomotor activity was also reduced slightly in some of these hybrids (Noor 1996b), the reduction in courtship intensity was substantially greater. Thus, I investigated both the genetics of sexual isolation and the genetics of this courtship dysfunction in male hybrids of *D. pseudoobscura* and *D. persimilis*.

MATERIALS AND METHODS

The wildtype strains of *D. pseudoobscura* and *D. persimilis* used in this study were collected in the summer of 1993 (see

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TABLE 1. Results of courtship of pure species males with *D. pseudoobscura* (*ps*) and *D. persimilis* (*per*) females. Medians (and ranges) are provided for estimates of courtship latency, courtship intensity, and courting time. The sample size used to determine courtship intensity is presented in subscript after the range. Latency measurements were derived from the pooled data of courtships to both *D. pseudoobscura* and *D. persimilis* females. Intensity measurements were taken from pairings of the males with females that had the most dissimilar genetic constitution (e.g., *D. pseudoobscura* males paired with *D. persimilis* females). Matings listed were those observed within five minutes after the initiation of courtship. Courting time indicates the time males that copulated spent performing courtship behaviors in pairings that resulted in copulation. "No-court" males were those that did not court females of either species during the 5-min observation period.

Male	No-court	Latency	Intensity	Mating *ps* females	Courting time	Mating *per* females	Courting time
1 *ps* Flag	0/54	10 (83)	$0.56\ (0.67)_{27}$	25/25	2 (35)	4/29	12 (138)
2 *per* MSH	1/58	13 (107)	$0.52\ (0.60)_{31}$	6/31	78 (90)	25/26	6 (88)
3 *per or*	4/76	23 (236)	$0.62\ (0.79)_{24}$	5/25	32 (52)	43/47	12 (144)

Noor 1995). They include Flagstaff (Flag), a *D. pseudoobscura* stock derived from three isofemale lines collected in Flagstaff, Arizona; Mather, a *D. persimilis* stock derived from three isofemale lines collected in Mather, California; and MSH, a *D. persimilis* stock derived from four isofemale lines collected at Mount Saint Helena, California. Since females derived from populations of *D. pseudoobscura* that are sympatric with *D. persimilis* display increased discrimination against *D. persimilis* males (Noor 1995), I did not use any *D. pseudoobscura* strains that were collected from sympatric populations. *D. persimilis*, however, is completely contained within the range of *D. pseudoobscura*. The following lists explain the mutant stocks that were obtained from the National Drosophila Species Resource Center.

Drosophila pseudoobscura: (1) *vermilion* (*v*): A stock homozygous for *vermilion* (bright red eye color), a recessive mutation on the left arm of the X-chromosome (XL) (1-84); (2) *sepia* (*se*): A stock homozygous for *sepia* (dark brown eye color), a recessive mutation on the right arm of the X-chromosome (XR) (1-156); (3) *Bare* (*Ba*): A stock heterozygous for the dominant bristle mutation *Bare* (missing bristles) on the second chromosome (2-62). This stock was derived by crossing the stock *yellow*; *Ba/Delta*; *orange* with the Flag strain above and selecting for F_1 offspring possessing *Bare*. For backcrosses to *D. persimilis*, F_1 hybrid females that displayed the mutant phenotype were selected and crossed to *D. persimilis* males.

Drosophila persimilis: *orange* (*or*): A stock homozygous for the recessive eye color mutation *orange* (bright red eyes when in the absence of other mutations) on the third chromosome located at 3-0.

All map locations were obtained from Anderson (1993) and Orr (1995). I used mutant strains whose males were as successful (or nearly so) at mating with conspecific females as were wildtype males, as determined by preliminary crosses. Several other mutations were rejected because they significantly decreased mating success. Further, only single-mutant lines were used because of our previous observation that multiple markers severely depresses viability (and hence, mating success or courtship intensity) of multiply marked hybrid flies (Noor and Coyne 1996).

Because of fixed inversion differences between the species on the X, second, and third chromosomes, the mutant markers segregate with large portions of the chromosomes. No marker was used on the fourth or fifth chromosomes, which comprise a total of about 15% of the genome, since no dominant *D. pseudoobscura* or recessive *D. persimilis* markers were available that did not affect mating success.

Stocks were kept and crosses were made at 21°C on cornmeal/Karo syrup/yeast/agar food, and carbon dioxide was used for anesthetization. Mount St. Helena strain *D. persimilis* or Flagstaff strain *D. pseudoobscura* females were used for every mating experiment. After eclosion, flies were kept in vials with others of the same sex for seven days to reach reproductive maturity. Flies were then transferred to individual vials and housed for one day to simplify crossing on the eighth day.

On the eighth day, single pairs of eight-day-old flies were aspirated without anesthesia into an 8-dram, food-containing vial and observed for five minutes after the initiation of courtship. If the male did not court the female for five minutes after initial confinement, the male was designated as a "noncourter" and discarded. The following measurements were taken on courting males: time to first male courtship (courtship latency), time from courtship initiation to copulation (courtship period), time the male spent performing courtship behaviors (courting time), and whether copulation occurred. The courtship period is greater than the courting time because males frequently break off courtship for short spells. Courtship behaviors by males included various combinations of the following behaviors: tapping the female's abdomen with the foretarsi, pursuing the female from behind, extending and vibrating one wing, lifting both wings and front legs in a display posture, licking the female's genitalia, and attempting to copulate (Brown 1964).

To control for environmental effects on courtship latency, equal numbers of males that were being compared to each other were tested on each day. A previous study has shown that male courtship latency and intensity in these species is identical regardless of whether they are presented with *D. pseudoobscura* or *D. persimilis* females (Noor 1996a). Hence, the courtship latencies presented in Table 1 are averaged over pairings with females of both species. To estimate the intensity of male courtship, I divided the courting time by the courtship period (Tompkins et al. 1980; Oguma et al. 1995). If the pair did not copulate, courting time was divided by five minutes (the total observation time). Since courtship intensity reflects the proportion of time the male spent performing courtship behaviors, short courtship periods can obscure the true intensity of male courtship. For example, a male courting for 100% of two seconds does not necessarily court with the same intensity as a male that courts for 100%

TABLE 2. Results of courtship of F_1 males with *D. pseudoobscura* (*ps*) and *D. persimilis* (*per*) females. F_1 males are listed by strain of mother first, then strain of father. The origin of their X-chromosome is also listed parenthetically. Measurements taken are the same as those in Table 1. Statistics compare the two classes of F_1 males for each pair of strains in the measurements taken in each of the columns.

Male	No-court	Latency	Intensity	Mating *ps* females	Courting time	Mating *per* females	Courting time
1 Flag (*ps*)							
Mather	0/64†	10 (44)‡	$0.52\ (0.76)_{36}$‡	25/25‡	2 (45)	21/39	39 (110)‡
2 Mather (*per*)							
Flag	8/64	41 (249)	$0.04\ (0.16)_{15}$	14/21	3 (14)	25/35	5 (40)
3 Flag (*ps*)							
MSH	0/50	12 (130)‡	$0.51\ (0.53)_{25}$‡	25/25†	2 (4)	5/25*	58 (63)†
4 MSH (*per*)							
Flag	3/50	31 (274)	$0.04\ (0.09)_{11}$	16/22	2 (6)	12/25	13 (67)
5 *v* (*ps*)							
MSH	0/50	10 (127)‡	$0.37\ (0.42)_{25}$‡	25/25‡	2 (46)†	7/25*	57 (107)‡
6 MSH (*per*)							
v	2/50	42 (252)	$0.08\ (0.28)_{20}$	12/24	6 (39)	15/24	11 (25)
7 *se* (*ps*)							
MSH	0/50	19 (264)	$0.47\ (0.52)_{25}$‡	25/25‡	2 (18)†	7/25*	55 (106)‡
8 MSH (*per*)							
se	4/50	34 (291)	$0.10\ (0.20)_{18}$	9/22	7 (17)	15/24	8 (31)
9 *Ba* (*ps*)							
MSH	0/50	10 (66)‡	$0.53\ (0.68)_{25}$‡	24/25†	2 (21)*	6/25	50 (99)†
10 MSH (*per*)							
Ba	5/50	46 (239)	$0.09\ (0.23)_{18}$	14/23	12 (48)	9/22	5 (13)
11 Flag (*ps*)							
or	0/50	8 (97)‡	$0.59\ (0.54)_{23}$‡	25/25*	2 (56)	7/25	37 (72)
12 *or* (*per*)							
Flag	1/50	19 (115)	$0.11\ (0.26)_{10}$	20/25	4 (30)	10/24	12 (27)

* $P \leq 0.05$; † $P \leq 0.01$; ‡ $P \leq 0.001$.

of five minutes. To avoid such biases in the estimates of courtship intensity, any matings that occurred less than 30 seconds (a time interval longer than any single courtship bout) after the beginning of courtship were excluded. To minimize the effects of this exclusion, only pairings with females that had the most dissimilar genetic constitution (e.g., backcross-to-*D. pseudoobscura* males paired with *D. persimilis* females) were analyzed, such that generally few exclusions were necessary. Since male courtship to females of either species is identical (Noor 1996a), this limitation should have no effect on the results. Additionally, for statistical comparisons of courting time (and all the courting time means given in Tables 1, 2), only the courting times of males who copulated were used. Hence, courting time in the tables and comparisons reflects the length of courtship that was necessary for the male to secure a copulation.

Comparisons of F_1 hybrid males were all between the males possessing an X-chromosome from *D. pseudoobscura* and the males possessing an X-chromosome from *D. persimilis*. Comparisons of backcross males presented in Table 3 were between males possessing a mutant marker and males from the same backcross lacking the marker (hence, between those possessing a particular chromosome arm from *D. pseudoobscura* and those possessing that same chromosome arm from *D. persimilis*).

Backcrosses to each species were performed to analyze the effect of the X-chromosome on mating success and courtship dysfunction. However, only backcrosses to *D. persimilis* were necessary to analyze autosomal effects since F_1 males copulated readily with *D. pseudoobscura* females but infrequently with *D. persimilis* females. Two-tailed probabilities were calculated by Mann-Whitney U-tests and Fisher's exact tests.

RESULTS

Pure Species Crosses

Nearly all intraspecific combinations resulted in immediate copulations, whereas few interspecific pairs mated (Table 1). Matings with conspecific females occurred after a much shorter courting time than matings with heterospecific females. Very few males did not court during the observation period.

F_1 Crosses

The results of the reciprocal F_1 crosses are presented in Table 2. F_1 hybrid males with an X-chromosome from *D. pseudoobscura* were consistently more successful than F_1 hybrid males with an X-chromosome from *D. persimilis* at mating with *D. pseudoobscura* females. The reciprocal trend was observed in matings with *D. persimilis* females, though the pattern was not consistently significant. However, striking differences exist between the two classes of F_1 males in courtship latency and intensity: hybrid males with an X-chromosome from *D. persimilis* exhibited an anomalously weak courtship intensity ($P < 0.001$ in every comparison) and always took longer to initiate courtship with females ($P < 0.001$ in five of six comparisons) than males with an X-chromosome from *D. pseudoobscura*. Also, the males with a *D. pseudoobscura* X-chromosome always courted females in the observation period, whereas several of the reciprocal hybrid males did not (Table 2, No-court column).

In many pairings with *D. pseudoobscura* females, there was no significant difference between the two classes of hybrid males in the courting time associated with successful cop-

TABLE 3. Results of courtship of backcross males with *D. pseudoobscura* (*ps*) and *D. persimilis* (*per*) females. Measurements taken are the same as those in Table 1. Statistics presented in this table compare backcross males possessing a mutant marker with those from the same backcross lacking that marker (hence, between those possessing a particular chromosome arm from *D. pseudoobscura* and those possessing that same chromosome arm from *D. persimilis*). In each comparison, the chromosome arm that came from *D. pseudoobscura* is listed first.

Male genotype	No-court	Latency	Intensity	Mating *ps* females	Courting time	Mating *per* females	Courting time
Backcross to *D. pseudoobscura*:							
1 XL *v* (*ps*)	0/100	11 (83)*	$0.54\ (0.65)_{47}$‡	47/50	2 (35)	11/50	23 (47)
2 XL + (*per*)	3/100	14 (223)	$0.21\ (0.79)_{42}$	42/50	2 (56)	11/47	15 (90)
3 XR *se* (*ps*)	4/101‡	26 (248)‡	$0.09\ (0.54)_{45}$	27/50	3 (83)	10/47	12 (47)
4 XR + (*per*)	19/101	47 (291)	$0.06\ (0.66)_{41}$	23/39	5 (25)	9/43	20 (103)
Backcross to *D. persimilis*:							
5 XL *v* (*ps*)	1/100	21 (257)	$0.54\ (0.74)_{25}$	35/49	4 (101)	25/50†	15 (56)
6 XL + (*per*)	1/100	23 (291)	$0.40\ (0.70)_{27}$	39/49	9 (109)	39/50	7 (111)
7 XR *se* (*ps*)	6/100	44 (266)	$0.32\ (0.76)_{40}$†	13/44‡	13 (8)	36/50	6 (91)
8 XR + (*per*)	1/100	38 (214)	$0.50\ (0.75)_{50}$	2/50	15 (4)	38/49	10 (56)
9 *Ba* (*ps*)	2/9	23 (47)	$0.48\ (0.66)_{4}$	0/4	NA	1/3	51 NA
10 + (*per*)	0/9	20 (264)	$0.35\ (0.38)_{5}$	2/5	26 (10)	2/4	22 (37)
11 + (*ps*)	2/100	18 (169)	$0.45\ (0.75)_{30}$	31/49	6 (190)	25/49	10 (76)
12 *or* (*per*)	4/100	16 (278)	$0.27\ (0.78)_{31}$	33/47	11 (117)	22/49	16 (84)

* $P \leq 0.05$; † $P \leq 0.01$; ‡ $P \leq 0.001$; NA = not applicable.

ulations, indicating that the difference in mating success with *D. pseudoobscura* females might result from the weak courtship of some males possessing a *D. persimilis* X-chromosome rather than female discrimination. In contrast, there was a large difference between the hybrid males in their courting time of *D. persimilis*, suggesting that there is probably an X-chromosome effect on the male character(s) that *D. persimilis* females use to identify conspecific males.

The differences between the two classes of F_1 males could result from X-chromosome or cytoplasmic effects. Cytoplasmic effects were observed in competition experiments in these species (Hutter and Rand 1995), so their possibility cannot be excluded. To confirm that the differences between F_1 hybrid males in courtship intensity and mating success did not result from cytoplasmic effects, I introgressed a *D. persimilis* (Mount St. Helena strain) cytoplasm into *D. pseudoobscura* (Flagstaff strain) by backcrossing hybrid females with *D. pseudoobscura* males for four generations. Twelve of the resultant cytoplasmic-introgression males were then crossed with females of each species. The males were all successful at mating with *D. pseudoobscura* females, six (half) were rejected by *D. persimilis* females, and the courtship intensity was similar to that observed in both pure-species crosses (median = 0.64), indicating that the anomalous courtship behavior and differences in mating success observed in the F_1 hybrid males probably result from nuclear gene effects.

Backcrosses Analyzing X-Chromosome Effects

Drosophila persimilis XL-chromosome arms in a predominantly *D. pseudoobscura* autosomal background caused males to court weakly (Table 3, lines 1–2, Intensity column). This chromosome arm probably contributes to the courtship dysfunction observed in F_1 males. The right arm of the X-chromosome had no effect in a *D. pseudoobscura* background. In contrast, in backcrosses to *D. persimilis*, *D. pseudoobscura* XR-chromosomes reduced male courtship intensity (lines 7–8, Intensity column). This result suggests that the observed hybrid male courtship dysfunction could be bidirectional: males with an X-chromosome from either species in the autosomal background of the other species court with reduced intensity. However, the *se* marker on the XR-chromosome only appeared in about one in seven backcross males, so the possibility that the mutation, *se*, itself had a stronger effect on viability in a *D. persimilis* background than in an F_1 hybrid background cannot be excluded. Weisbrot (1963) also noted much greater inviability in progeny of backcrosses to *D. persimilis* than to *D. pseudoobscura*. The XL marker (*v*) appeared in the expected 50% of backcross males to either species, as did the *se* marker in backcrosses to *D. pseudoobscura*.

In the backcrosses to *D. pseudoobscura*, both arms of the X-chromosome had a significant effect on courtship latency, but the effect of the right arm was much larger than that of the left arm (Table 3, lines 1–4, Latency column). A large effect of the right arm of the X-chromosome was also observed in the proportion of males that did not court in the observation period (lines 1–4, No-court column). Hence, I conclude that the increased courtship latency of hybrid males is not produced by the same gene incompatibilities as the reduced courtship intensity.

Tests of mating success showed X-chromosome components of sexual isolation with both *D. pseudoobscura* and *D. persimilis*, though the genes contributing to sexual isolation from females of the two species were on different chromosome arms (Table 3, lines 5–6, Mating *per* females column; lines 7–8, Mating *ps* females column). These X-chromosome effects were observed in backcrosses to *D. persimilis*, but no significant effects of the X-chromosome were detected on mating success with females of either species in backcrosses to *D. pseudoobscura* (lines 1–4, Mating *ps* females and Mating *per* females columns). The differences between these

backcrosses were directly tested for significance using a binomial test of proportions ($Z = 2.36$, $P < 0.02$ for XR difference in mating success with *D. pseudoobscura* females and $Z = 2.12$, $P < 0.05$ for XL difference in mating success with *D. persimilis* females). The significant differences suggest that the X-chromosome is interacting with the autosomes or Y-chromosome to affect male mating success, since only the autosomes and Y-chromosome differ between the backcrosses.

These differences in mating success in the two backcross directions might have been affected by differences in courtship intensity. However, the intensity difference should have reduced the mating success of *se* males with *D. pseudoobscura* females. Instead, these backcross males were significantly more successful at mating with *D. pseudoobscura* females than males not possessing the *se* marker (see Table 2.3, lines 7–8, Mating *ps* female column). Hence, the results provide preliminary evidence for epistasis among loci affecting mating success in *D. pseudoobscura* and possibly *D. persimilis*. No significant differences in courting time between the X-chromosomes of the two species were observed in any of the backcrosses.

Backcrosses Analyzing Autosomal Effects

In the backcrosses to *D. persimilis* involving a marked second-chromosome from *D. pseudoobscura*, almost no offspring bore the marker *Bare*. Apparently, this *D. pseudoobscura* marker was linked to alleles that cause extreme meiotic drive or inviability in a *D. persimilis* background. In a single food-containing bottle set up with 14 *D. persimilis* males and 14 F_1 females possessing the *Bare* marker, 171 offspring hatched, and only seven of these (all female) carried the marker. In contrast, when 14 *D. pseudoobscura Bare* males were crossed to 14 *D. persimilis* females to produce the F_1 females, exactly half of the 42 offspring bore the marker. Hence, the inviability does not appear to be related to the marker itself.

The analysis of the second-chromosome is thus not as thorough as would have been desired. Still, from the limited information derived, the second-chromosome appears not to affect courtship intensity greatly, and it does not have a very large effect on mating success with *D. pseudoobscura* females (Table 3, lines 9–10).

Loci linked to the third chromosome marker also had little or no effect on mating success with females of either species or on courtship intensity (Table 3, lines 11–12). In fact, males with a heterospecific third-chromosome had non-significantly greater mating success with females of either species.

It is surprising that the two autosomes investigated have almost no effect on sexual isolation when an autosomal component is suggested by the backcrosses examining the effect of the X-chromosome. I can only conclude that the fourth, fifth, or Y-chromosome has a large effect on mating success; that regions on the second or third chromosome that are not linked to the markers have a large effect on mating success; or that mating success results from a more complex interaction of loci than a simple backcross analysis can reveal.

Discussion

This study yields four major observations. First, F_1 hybrid males with a *Drosophila pseudoobscura* father and *D. persimilis* mother display an anomalously weak courtship when presented with potential mates. Second, this courtship deficiency appears to result from interactions between the left arm of the *D. persimilis* X-chromosome and the *D. pseudoobscura* autosomes or Y-chromosome. Third, the left arm of the X-chromosome possesses one or more factors that contribute to hybrid male mating success with *D. persimilis* females, and the right arm of the X-chromosome possesses factors that contribute to mating success with *D. pseudoobscura* females. Fourth, some preliminary evidence suggests that these genetic factors act epistatically with the autosomes (or Y-chromosome) to produce the male phenotypes that females accept, particularly in *D. pseudoobscura*.

The results presented here bear on the questions on the evolution of sexual isolation described in the introduction. I will discuss these questions in turn.

Are There Patterns to the Genetic Basis of Sexual Isolation, such as a Disproportionately Large X-chromosome Effect?

The X-chromosome accounts for nearly half the *D. pseudoobscura* genome, as determined by recombination (Anderson 1993; Orr 1995). In contrast, in the backcross analyses presented here, the effect of the X-chromosome on the proportion of copulations is less than half of the difference between the proportion of copulations in intraspecific and interspecific crosses. Similarly, in studies of the sexual isolation of male hybrids of *D. simulans* and *D. mauritiana* (Coyne 1996) and the semispecies of *D. paulistorum* (Ehrman 1961), the mating success of male hybrids was not disproportionately controlled by X-linked factors. Hence, the sexual isolation of *Drosophila* species does not appear to result from the fixation of advantageous recessive alleles frequently affecting male characters (Charlesworth et al. 1987). Other taxa in which the males are heterogametic also do not show large X-effects to assortative mating or mating signals, but surprisingly, Lepidoptera do appear to have frequent sex-linkage of such characters (for review, see Ritchie and Phillips, *in press*). Studies of mating signals in birds will be instructive as to whether this discrepancy is specific to Lepidoptera or a consequence of female heterogamety.

Do Genes that Confer Sexual Isolation Act Epistatically?

The results presented here suggest that there are epistatic interactions among the genes that produce the male character(s) that females use to identify conspecific mates. Zouros (1981) noted some epistasis among the autosomes that had a very minor effect on the sexual isolation of *D. arizonae* and *D. mojavensis*, but other studies have not detected epistasis in the sexual isolation of males in other species pairs (e.g., Coyne 1996). This difference could result from the crossing technique: genetic studies of sexual isolation that use backcrosses to a single species preclude the detection of epistasis except between chromosome markers that are used together. The bi-directional backcross technique used here characterizes X-chromosome effects in more detail and is

more sensitive for detecting some interactions since the X is held constant but the autosomes and Y-chromosome are varied.

The finding of epistasis in the loci conferring sexual isolation is not unexpected, as the genetic changes in these species that ultimately caused their sexual isolation occurred in diverging genetic backgrounds. Hence, although these loci seem to act epistatically, they may have resulted from the accumulation of alleles with additive effects within the species. Theoretical models of speciation generally assume that such changes are largely additive (e.g., Liou and Price 1994; Turner and Burrows 1995). Invoking epistasis could alter the predictions of some such models.

Do Related Species Use Allelic Variants of the Same Loci to Recognize Conspecifics?

The relative effects of the X-chromosome arms on sexual isolation of males from *D. pseudoobscura* and *D. persimilis* females differ. This finding suggests that different loci produce the male characters that females of each species use to identify conspecific mates. The frequent observation of unidirectional species discrimination also suggests that related species do not commonly use allelic variants of the same loci to identify conspecifics (for review, see Kaneshiro 1989).

What Phenotypic Characters Are Involved in the Sexual Isolation of Drosophila Species?

By examining the correlation between the genetics of hybrid male sexual isolation and the genetic basis of phenotypic differences between species, we can identify characters which might contribute to that sexual isolation. Two phenotypic character differences have been shown to contribute to sexual isolation in *Drosophila* species: cuticular hydrocarbon profile and male courtship wingbeat (e.g., Coyne et al. 1994; Kyriacou and Hall 1982; Tomaru et al. 1995). Although *D. pseudoobscura* and *D. persimilis* differ in cuticular hydrocarbon composition, the genetic basis of this difference is incongruent with the genetics of sexual isolation in these species (Noor and Coyne 1996), suggesting that females do not use this difference to identify conspecific males. These species differ in several aspects of their male courtship wingbeat (Waldron 1964; Ewing 1969), and I am currently studying the genetic basis of the wingbeat differences to evaluate their role in the sexual isolation of these species.

The finding of a large difference between the two classes of F_1 males in courtship intensity was unexpected. This difference in courtship intensity and mating success is not a simple product of a difference in locomotor activity level (e.g., Meffert and Bryant 1992; see Hall 1994, for review). Hybrid males of Flagstaff strain *D. pseudoobscura* and Mount St. Helena strain *D. persimilis* bearing a *D. persimilis* X-chromosome are about 80% as active as the reciprocal males (Noor 1996b). However, the *D. persimilis* X-chromosome males court at about 10% of the intensity of the reciprocal males (Table 2, lines 3–4). This difference suggests that the observed deficiency in courtship intensity is not a simple result of reduced locomotor activity level.

This reduced fitness of the heterogametic sex appears to be a behavioral manifestation of Haldane's Rule (Haldane 1922): if one hybrid sex is sterile or inviable, it tends to be the heterogametic sex. Reduced courtship has also been observed in hybrid males of the *Drosophila athabasca* races (Yoon 1991). Similar failures of heterogametic hybrids to court or copulate with potential mates occur in hybrid females of two host-races of the fall armyworm, *Spodoptera frugiperda* (Pashley and Martin 1987), and in hybrid females of the neotropical butterflies *Anartia fatima* and *A. amathea* (Davies et al., *in press*). It is not unusual that complex behaviors, such as courtship, are prone to hybrid anomalies since physiological processes, such as gamete development, are frequently affected. Further research is necessary to determine if this barrier to gene exchange could be relevant to the speciation process itself or is merely a byproduct of genetic divergence that occurs after speciation is complete.

Though this courtship dysfunction greatly reduced the intensity with which males displayed to females, these males were sometimes able to mate successfully, suggesting that, even in the absence of the existing hybrid male sterility, it would not be a very effective barrier to gene exchange in these species. Indeed, the strength of sexual isolation by female mate discrimination in these species was substantially greater at preventing copulations, as evidenced by the occasional greater mating success of the "dysfunctional" hybrids than their counterparts (e.g., see Table 2, Mating *per* females column). Nevertheless, this courtship dysfunction did reduce overall mating success, if by no other means than by preventing several males from courting altogether (see Table 2, No-court column), and the effects of such courtship dysfunction in hybrids should be considered in genetic studies of sexual isolation. If I inferred the nature of sexual isolation solely from the mating success of the reciprocal crosses, I would have incorrectly concluded that there was a large effect of the X-chromosome in mating success with *D. pseudoobscura*, and a very small effect of the X-chromosome on mating success with *D. persimilis*. Some previous studies of sexual isolation could have suffered this artifactual result, particularly experiments using the "female-choice" or "multiple-choice" designs. Male vigor has been suggested as being important for mating success in several other studies of these species as well (e.g., Anderson et al. 1979; Harmsen and Clark 1987). It has been further suggested that differences in male courtship intensity or female mating propensity could also complicate the results of empirical studies of founder effect speciation (Barton and Charlesworth 1984). Evaluating the number of courtship bouts or attempted copulations could be satisfactory substitutes for observing the proportion of time the male spent courting, but some attempt must be made at quantifying the intensity of hybrid male courtship in genetic studies of sexual isolation.

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Literature Cited

Anderson, W. W. 1993. Linkage map of *Drosophila pseudoobscura*. P. 3.252 *in* S. J. O'Brien, ed. Genetic maps: locus maps of complex genomes. Cold Spring Harbor Laboratory Press, Plainview, NY.

Anderson, W. W., L. Levine, O. Olvera, J. R. Powell, M. E. de la Rosa, V. M. Salceda, M. I. Gaso, and J. Guzman. 1979. Evidence for selection by male mating success in natural populations of *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci., USA 76:1519–1523.

Barton, N. H., and B. Charlesworth. 1984. Genetic revolutions, founder effects, and speciation. Ann. Rev. Ecol. Syst. 15:133–164.

Brown, R. G. B. 1964. Courtship behaviour in the *Drosophila obscura* group. I.: *D. pseudoobscura*. Behaviour 23:61–106.

Charlesworth, B., J. A. Coyne, and N. H. Barton. 1987. The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. 130:113–146.

Coyne, J. A. 1992a. Genetics and speciation. Nature 355:511–515.

———. 1992b. Genetics of sexual isolation in females of the *Drosophila simulans* species complex. Genet. Res. Camb. 60:25–31.

———. 1996. Genetics of sexual isolation in male hybrids of *Drosophila simulans* and *D. mauritiana*. Genet. Res. Camb. 68:211–220.

Coyne, J. A., A. P. Crittenden and K. Mah. 1994. Genetics of a pheromonal difference contributing to reproductive isolation in *Drosophila*. Science 265: 1461–1464.

Davies, N., A. Aiello, J. Mallet, A. Pomiankowski, and R. E. Silberglied. 1997. Speciation in two neotropical butterflies: extending Haldane's rule. Proc. R. Soc. Lond. B Biol. Sci. In press.

Ehrman, L. 1961. The genetics of sexual isolation in *Drosophila paulistorum*. Genetics 46:1025–1038.

Ewing, A. W. 1969. The genetic basis of sound production in *Drosophila pseudoobscura* and *D. persimilis*. Anim. Behav. 17: 555–560.

Haldane, J. B. S. 1922. Sex ratio and unisexual sterility in hybrid animals. J. Genet. 12:101–109.

Hall, J. C. 1994. The mating of a fly. Science 264:1702–1714.

Harmsen, R., and D. R. Clark. 1987. The effects of inbreeding and ventilation on mating behavior in *Drosophila pseudoobscura*. Behav. Prog. 15:181–190.

Hutter, C. M., and D. M. Rand. 1995. Competition between mitochondrial haplotypes in distinct nuclear genetic environments: *Drosophila pseudoobscura vs. D. persimilis*. Genetics 140: 537–548.

Kaneshiro, K. Y. 1989. The dynamics of sexual selection and founder effects in species formation. Pp. 279–296 *in* L. V. Giddings, K. Y. Kaneshiro, and W. W. Anderson, eds. Genetics, speciation and the founder principle. Oxford Univ. Press, New York.

Kyriacou, C. P., and J. C. Hall. 1982. The function of courtship song rhythms in *Drosophila*. Anim. Behav. 30: 794–801.

Liou, L. W., and T. D. Price. 1994. Speciation by reinforcement of premating isolation. Evolution 48:1451–1459.

Mayr, E. 1963. Animal species and evolution. Belknap Press, Cambridge, MA.

Meffert, L. M., and E. H. Bryant. 1992. Divergent ambulatory and grooming behavior in serially bottlenecked lines of the housefly. Evolution 46:1399–1407.

Noor, M. A. 1995. Speciation driven by natural selection in *Drosophila*. Nature 375:674–675.

———. 1996a. Absence of species discrimination in *Drosophila pseudoobscura* and *D. persimilis* males. Anim. Behav. 52:1205–1210.

———. 1996b. Activity level of *Drosophila pseudoobscura* and *D. persimilis* hybrid males. Drosoph. Inf. Serv. 77:51–52.

Noor, M. A. F., and J. A. Coyne. 1996. Genetics of a difference in cuticular hydrocarbons between *Drosophila pseudoobscura* and *D. persimilis*. Genet. Res., Camb. 68:117–123.

Oguma, Y., S. Wen, M. Tomaru, H. Matsubayashi, and T. Peng. 1995. Reproductive isolation between *Drosophila lini* and its siblings. Jpn. J. Genet. 70:311–320.

Orr, H. A. 1995. A new linkage map of the *D. pseudoobscura* X chromosome. *Drosophila* Inf. Serv. 76:127–128.

Pashley, D. P., and J. A. Martin. 1987. Reproductive incompatibility between host strains of the fall armyworm (Lepidoptera: Noctuidae). Ann. Ent. Soc. Am. 80:731–733.

Ritchie, M. G. and S. D. F. Phillips. In press. The genetics of sexual isolation. *In* D. Howard and S. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, Oxford.

Tan, C. C. 1946. Genetics of sexual isolation between *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics 31:558–573.

Tomaru, M., H. Matsubayashi, and Y. Oguma. 1995. Heterospecific inter-pulse intervals of courtship songs elicit female rejection in *Drosophila biauraria*. Anim. Behav. 50: 905–914.

Tompkins, L., J. C. Hall, and L. M. Hall. 1980. Courtship-stimulating volatile compounds from normal and mutant *Drosophila*. J. Insect Physiol. 26:689–697.

Turner, G. F., and M. T. Burrows. 1995. A model of sympatric speciation by sexual selection. Proc. R. Soc. Lond. B Biol. Sci. 260:287–292.

Waldron, I. 1964. Courtship sound production in two sympatric sibling *Drosophila* species. Science 144:191–193.

Weisbrot, D. G. 1963. Studies on differences in the genetic architecture of related species of *Drosophila*. Genetics 48:1121–1139.

Yoon, C. K. 1991. Molecular and behavioral evolution in the semispecies of *Drosophila athabasca*. Ph.D. diss., Cornell Univ., Ithaca, NY.

Zouros, E. 1981. The chromosomal basis of sexual isolation in two sibling species of Drosophila: *D. arizonensis* and *D. mojavensis*. Genetics 97:703–718.

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