POSTMATING-PREZYGOTIC ISOLATION IS NOT AN IMPORTANT SOURCE OF SELECTION FOR REINFORCEMENT WITHIN AND BETWEEN SPECIES IN DROSOPHILA PSEUDOOBSCURA AND D. PERSIMILIS

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Abstract.--Most work on adaptive speciation to date has focused on the role of low hybrid fitness as the force driving reinforcement (the evolution of premating isolation after secondary contact that reduces the likelihood of matings between populations). However, recent theoretical work has shown that postmating, prezygotic incompatibilities may also be important in driving premating isolation. We quantified premating, postmating-prezygotic, and early postzygotic fitness effects in crosses among three populations: Drosophila persimilis, D. pseudoobscura USA (sympatric to D. persimilis), and D. pseudoobscura Bogotá (allopatric to D. persimilis). Interspecific matings were more likely to fail when they involved the sympatric populations than when they involved the allopatric populations, consistent with reinforcement. We also found that failure rate in sympatric mating trials depended on whether D. persimilis females were paired with D. pseudoobscura males or the reverse. This asymmetry most likely indicates differences in discrimination against heterospecific males by females. By measuring egg laying rate, fertilization success and hatching success, we also compared components of postmating-prezygotic and early postzygotic isolation. Postmating-prezygotic fitness costs were small and not distinguishable between hetero- and conspecific crosses. Early postzygotic fitness effects due to hatching success differences were also small in between-population crosses. There was, however, a postzygotic fitness effect that may have resulted from an X-linked allele found in one of the two strains of D. pseudoobscura USA. We conclude that the postmating-prezygotic fitness costs we measured probably did not drive premating isolation in these species. Premating isolation is most likely driven in sympatric populations by previously known hybrid male sterility.

Key words.—Hybridization, postzygotic, reinforcement, speciation, X-linked.

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Biologists have long been interested in the role of adaptation in the formation of species. When two allopatric populations come into secondary contact, reduced hybrid fitness can lead to adaptive divergence of mating preferences in a process known as reinforcement (Dobzhansky 1940). This adaptive evolution of premating isolation may be necessary for speciation whenever there is continued gene flow or upon secondary contact. Recent theory suggests that premating isolation can also be driven by other sources of selection upon secondary contact (Servedio 2001; Kirkpatrick and Revigné 2002). One such source is a fitness reduction occurring after mating but before zygote formation (postmating-prezygotic isolation or PPI). Many examples of fitness costs at this stage have been reported (reviewed in Servedio 2001) including increased female mortality (before giving birth or laying eggs) and lower fertility of females that mate with heterospecifics. The later mechanism may be due to males not stimulating ovulation or fertilization; ineffective transfer, storage, or passage of heterospecific sperm through the female; or incompatibilities between sperm proteins and receptors in the egg. Several authors have suggested costs at this stage may be important in speciation (e.g., Howard and Gregory 1993; Markow 1997; Price et al. 2001; Knowles et al. 2004).

Using the methodology of Barton and Turelli (1991), Servedio (2001) compared the strength of selection placed on diverging preferences by PPI and viability selection against hybrids. The haploid model included all fitness costs due to heterospecific matings in the postmating-prezygotic and post-zygotic stages. The analyses were based on a set of simplifying assumptions, including that the number of sets of loci causing incompatibilities, and the strength of selection af-

fecting each set, were equal across incompatibility types. The model predicted that PPI can be as strong as reduced hybrid fitness in driving premating isolation. However, because of the simplifying assumptions of the model, empirical comparisons of the strength of selection arising from PPI and low hybrid fitness are necessary to thoroughly understand the forces involved in reinforcement.

Based on the clear prediction that PPI can drive premating isolation in a way analogous to reinforcement and the fact that PPI is apparently common, we measured components of premating isolation, PPI, and early postzygotic isolation. We chose to use Drosophila pseudoobscura (from two allopatric populations) and *D. persimilis* (sympatric with one of the *D*. pseudoobscura populations) because of the substantial amount of work on premating isolation and postzygotic isolation in this species pair. Earlier work had shown evidence for hybridization in the wild (Dobzhansky 1973; Powell 1983), no evidence of discrimination by courting males (against heterospecifics Mayr 1946; Noor 1996), substantial premating isolation via female mate discrimination in sympatry (Noor 1995), as well as postzygotic fitness reductions in the form of sterility in hybrid males (Dobzhansky 1936; Prakash 1972) and F₂ hybrid breakdown (Orr 1987).

Two previous studies have examined the postmating-prezygotic stage in *D. pseudoobscura* and *D. persimilis* but did not test the prediction that PPI can drive premating isolation. Dobzhansky (1947) showed that females mated to heterospecifics did not lay fertile eggs for as long nor did they produce as many offspring as those mated to conspecifics. This study did not report either the fertilization rate or the hatching success of each type of cross. Without these two measures it is difficult to separate PPI from postzygotic isolation. By reporting only the number of days of egg laying that produced adult offspring and the number of adult offspring, the study lumps a component of PPI (fertilization success) with components of postzygotic isolation (larval and pupal survival, and eclosion success). In another study Dixon et al. (2003) demonstrated weak conspecific sperm precedence between populations of D. pseudoobscura (conspecific sperm out-fertilized heterospecific sperm). This may reduce the effectiveness of both PPI and postzygotic isolation in causing reinforcement (Marshall et al. 2002). However, because females discriminate strongly against heterospecific males in these species (Noor 1995), conspecific sperm precedence does not seem to have been important in reducing selection for premating isolation. Neither of these studies test whether PPI can be strong enough to drive the evolution of premating isolation.

By comparing the three kinds of heterotypic crosses with homotypic crosses, we tested whether PPI can be as strong as an early components of postzygotic isolation. Because hybrid male sterility and F_2 hybrid breakdown are well documented in these species, we focused on inviability early in the postzygotic stage, a component of postzygotic isolation that is seldom measured. Our goal was to complement earlier studies and provide a conclusive assessment of the role of PPI relative to other sources of isolation in selecting for reinforcement in these species.

MATERIALS AND METHODS

Fly Stocks and Rearing

Five of the six strains were obtained from the Tucson Stock Center (http://stockcenter.arl.arizona.edu/). Unique numbers were assigned to each strain and used instead of species names to reduce experimenter bias (shown in parentheses after stock number). These numbers were also used to keep track of interstrain crosses (see "Interstrain cross" below). Drosophila persimilis strains were 14011-0111.35 (1) from Mt. San Jacinto, California and 14011-0111.42 (2) from Port Coquitlam, British Columbia, Canada. Drosophila pseudoobscura strains were 14011-0121.35 (3) from Bogotá, Colombia (6/1960, A. Hunter), 14011-0121.42 (4) from Mather, California (1959), and 14011-0121.81 (6) from Port Coquitlam, British Columbia, Canada (A. Beckenbach 7/1982). For consistency with previous studies we refer to strains from Canada and the United States as USA strains. One strain came from M. Noor, Louisiana State University, Baton Rouge, LA (his "D. pseudoobscura Bogotana Susa #6", our 7) from Bogotá, Colombia.

Flies were reared on standard cornmeal-yeast-agar medium in either 8 dram plastic vials or in 200 ml plastic bottles and kept at 21°C on a 12 hour light:dark cycle. All strains were maintained in the laboratory for at least three generations before crosses were done. Adults were transferred to fresh food twice a week, allowed to lay eggs for two to three weeks, and containers with emerging flies were discarded two to three weeks after flies began emerging.

Crossing Protocol

In all matings, virgins were collected from cleared vials/ bottles and aged from six to eight days in same-sex containers before mating. Light CO_2 anesthesia was used only for sexing flies while collecting virgins. At all other times flies were moved by aspiration.

Interstrain crosses

To increase the robustness of experimental strains, individuals used in the experiments were F1 offspring of crosses between virgins from each of the two strains within a subspecies (D. pseudoobscura USA, D. pseudoobscura Bogotá, and D. persimilis). This was done both ways with respect to sex. In other words, 30 males of strain 1 were crossed with 30 females from strain 2 to produce offspring, referred to as 1,2 persimilis offspring. Similarly, 30 males of strain 2 were crossed with 30 females from strain 1 producing 2,1 persimilis offspring. Strains 3 and 7 were crossed in a similar way to produce 3,7 and 7,3 Bogotá offspring. Finally, strains 4 and 6 were crossed to produce 4,6 and 6,4 USA offspring. The direction of the interstrain cross turned out to be important, thus we use these two number designations to keep track of interstrain cross direction below. Mated flies were given new food bottles every three to four days to standardize larval density.

Experimental crosses

To get a baseline for the fitness effects that we measured, we performed crosses within each of the interstrain crosses (homotypic crosses; e.g., 1,2 males with 1,2 females and 2,1 males with 2,1 females). Three sets of crosses between species or between subspecies were performed in a similar way (heterotypic crosses; sympatric D. persimilis \times D. pseudoobscura USA, and allopatric D. pseudoobscura Bogotá \times D. pseudoobscura USA and D. pseudoobscura Bogotá \times D. persimilis). All of these cross types were done in two directions with respect to sex and interstrain cross. For example, the sympatric crosses between D. persimilis and D. pseudoobscura USA involved 1,2 males crossed with 4,6 females, 4,6 males crossed with 1,2 females, 2,1 males crossed with 6,4 females, and 6,4 males crossed with 2,1 females. (Thus, due to labor restrictions at the time of mating, only half of all possible combinations between the interstrain crosses were performed. For example crosses between 1,2 lines and 6,4 lines and between 2,1 lines and 4,6 lines were not done.) Because certain kinds of mating trials failed more often than others, more of these kinds of trials were performed (see Table 1). The term "mating type" throughout the rest of the paper refers to which cell of Table 1 a mating falls into.

Mating Protocol

Matings and oviposition were performed in mating chambers constructed from the bottom of plastic petri plates $(60 \times 15 \text{ mm}; \text{Falcon #35-1007})$ and a top from 100 ml plastic beakers (with small holes for air; Oxford Tri-pour #8889-206200). The petri plate had been filled with 6 ml of hard apple-juice agar on top of which a drop of yeast concentrate was added after cooling. This provided a substrate onto which TABLE 1. Number of mating trials of each type. Diagonal (**in bold**) shows within species homotypic trials, off the diagonal shows heterotypic trials. The two numbers represent each of the interstrain crosses. Sample sizes are higher in mating trials with higher mating failure rates.

	Female		
	D. pseudoobscura		
	Bogotá	USA	D. persimilis
Male			
Bogotá	13, 13	11, 10	17, 18
USA	16, 15	14, 10	16, 15
D. persimilis	11, 8	24, 24	11, 10

TABLE 2. Number of successful matings of each type. Diagonal (**in bold**) shows within species homotypic trials, off the diagonal shows heterotypic trials. The two numbers represent each of the interstrain crosses.

	Female			
	D. pseud	D. pseudoobscura		
	Bogotá	USA	D. persimilis	
Male				
Bogotá USA D. persimilis	9, 10 15, 11 6, 7	10, 7 12, 10 4, 2	6, 8 7, 7 8, 6	

females would oviposit, and on which eggs and larvae could be counted.

Individual virgin males from the interstrain crosses were placed in these chambers about 24 hours in advance of matings to increase their interest in mating (after being held for days with only males). Individual virgin females were then added to the chamber at the start of a one hour observation period. Pairs failing to mate during this time were discarded. Pairs in which males remained mounted for greater than two minutes were considered to have mated. Males were removed from the chambers and discarded after they dismounted the female. The number of successful matings are shown in Table 2. Females were allowed to oviposit on plates and were transferred to fresh plates every 24 hours for four days.

Measuring Fitness Components

Mating failure rate (*Failed matings/Total mating trials*) for each type of mating was used to compare premating isolation between different heterotypic matings.

Eggs were counted immediately after females were transferred to new plates (see Table 3 for counts). Plates were lidded and stored in rearing chambers for 48 hours, when they were frozen. A pilot study showed 48 hours to be enough time for greater than 90% of eggs to hatch. Unhatched eggs were counted and categorized into either unfertilized (white with characteristic opacity) or fertilized but unhatched (brown with some evidence of cell division). Using egg color may potentially misclassify some eggs, however, we have found no evidence for this in our own results or in the literature; this is a generally accepted rule of thumb. Furthermore, in our own examination of the four cases where females laid eggs but none hatched, all eggs were white; this implies that when sperm transfer fails, white eggs result.

Fertilization failure rate (*White eggs/Total eggs*) was used to compare postmating-prezygotic isolation between different heterotypic matings. This estimate excludes effects of heterotypic matings on female longevity beyond the first four days after mating (see Discussion). Female fertility (number of eggs laid) was only affected by her population type and not by mating type, so we do not report fertility.

Developmental failure rate (*Brown eggs/(Total eggs – White eggs*)) was used to compare early postzygotic isolation between different heterotypic matings. This is an early portion of the "postzygotic" life cycle stage not covered by other studies using these species that demonstrated hybrid male sterility and F_2 hybrid breakdown.

Statistical analysis

Mating failure (nominal discreet variable: failed or succeeded) was analyzed with logistic regression in three ways. First, to determine whether failure was associated with interstrain cross and/or mating type, we regressed mating failure on interstrain cross direction, mating type, and the interaction of these factors. In a second analysis, to determine whether mating failure was associated with mating type and/ or the direction of heterotypic matings with respect to sex, we regressed mating failure on mating type (ignoring direction with respect to sex) and mating type nested within mating type (ignoring direction). "Mating type (ignoring direction)" ignores the direction in which a cross was done with respect to sex. This means that, for example, D. pseudoobscura Bogotá male \times D. persimilis female and D. persimilis male \times D. pseudoobscura Bogotá female crosses are considered together. The nested factor tests the effect of direction on the association, whereas mating type (ignoring direction) tests for difference among mating types independent of direction with respect to sex. This analysis takes into account that homotypic matings only have one direction. Finally, we asked if mating failure was associated with the population of origin of males, females, or the interaction of these two variables. This last analysis helps to separate influences of male and female population of origin and their interaction. We report Likelihood ratio χ^2 statistics (calculated using JMP 5.0) for each effect.

For the analysis of fertilization failure and developmental failure, we used one-way ANOVA to test the effect of mating

TABLE 3. Number of eggs counted from all females of each type. Diagonal (**in bold**) shows within species homotypic trials, off the diagonal shows heterotypic trials. The two numbers represent each of the inter-strain crosses.

	Female		
	D. pseudoobscura		
	Bogotá	USA	D. persimilis
Male			
Bogotá USA D. persimilis	768, 931 1081, 1067 603, 595	367, 329 787, 786 90, 211	838, 794 1007, 736 909, 896



FIG. 1. Premating isolation demonstrated by mating failure rate. Panel labels show cross type (abbreviations: M, male; F, female; Dpe, *D. persimilis*, Bog, *D. pseudoobscura* Bogotá; USA, *D. pseudoobscura* USA). The first two rows show heterotypic matings, whereas the bottom row shows homotypic matings. Filled and open dots represent two directions of interstrain cross (arbitrarily, "A" indicates males from high numbered strain were mated to females of low numbered strain, "B" indicates the reverse). Dashed reference lines show the mean of homotypic matings (bottom panels; 0.29).

type (including direction with respect to sex and interstrain cross direction; using JMP 5.0). Figures were plotted using the Lattice package in R (version 1.9.0).

RESULTS

Premating Isolation

Figure 1 shows differences in mating failure rate between mating types. If classical reinforcement is the cause of the premating isolation, we would predict that mating failure rate would be highest in the sympatric heterotypic mating type (DpeUSA). Ignoring interstrain cross direction and direction with respect to sex for the moment, this is indeed what we see in Figure 1. The mating failure rate is 0.68 overall for DpeUSA. We also see that the mating type with the next highest failure rate is the interspecific but allopatric mating type (BogDpe, 0.41 failure rate), as expected. The intraspecific, allopatric mating type has the lowest overall mating failure rate (BogUSA, 0.13 failure rate).

Figure 1 makes clear that while interstrain cross direction has no effect (open and filled dots; L-R χ^2 (df = 1) = 0.405, P = 0.52), mating type significantly affects mating failure rate (L-R χ^2 (df = 1) = 76.853, P < 0.00001). This indicates significant premating isolation. The significant mating type effect can be further broken down to look at the separate effects of direction of cross with respect to sex and mating type (ignoring direction). Both of these factors are signifi-



FIG. 2. Interaction profile plot showing the probability of successful matings between members of each species or population. These plots use the logistic regression model estimating the effect of male and female population of origin, and the interaction between these factors on whether or not mating failed to occur. They show how the effect of one variable (male or female population of origin) is predicted to change with changes in the other variable (female or male population of origin). Lines are dotted for USA, solid for Bog, and dashed for Dpe. The left panel shows the predicted probability of males of each kind (shown as separate lines) mating with each kind of females (x axis) and the right panel shows the same thing for each kind of female.

cantly associated with mating failure (Direction: L-R χ^2 (df = 3) = 12.554, *P* < 0.0057; Mating type (ignoring direction): L-R χ^2 (df = 1) = 51.946, *P* < 0.00001).

Figure 2 makes one additional point not made in previous analyses. There was a significant interaction between male and female population of origin (L-R χ^2 (df = 4) = 33.761, P < 0.00001). The interaction is primarily due to the reduced probability of mating of *D. persimilis* males with *D. pseudoobscura* USA females (see Fig. 2) relative to other types of mates. Interestingly, matings between *D. persimilis* females and *D. pseudoobscura* USA males were more successful and do not contribute significantly to the interaction. This asymmetry indicates that either *D. pseudoobscura* USA females are more discriminating than *D. persimilis* females against heterospecific males or that *D. persimilis* males court *D. pseudoobscura* USA females less vigorously than conspecific (and *D. pseudoobscura* Bogotá) females.

Postmating-Prezygotic Isolation

Figure 3 shows that there was no effect of mating type (including direction with respect to sex and interstrain cross direction) on fertilization failure rate (F(df = 17) = 1.25, P = 0.24). The grand mean fertilization failure rate across mating types was a very low 0.02. The figure also shows that interstrain cross direction had no effect on fertilization failure rate. Postmating-prezygotic isolation is also no stronger be-





FIG. 3. Postmating-prezygotic isolation demonstrated by fertilization failure rate. Panel and axis labels are as in Figure 1. Each dot represents the mean failure rate of a single female. Filled and open dots represent two directions of interstrain cross.

FIG. 4. Postzygotic isolation demonstrated by developmental failure rate. Panel labels show cross type with notation as in Figure 1.

tween species than it is between populations within a species and no stronger in sympatric than allopatric population pairs.

Early Postzygotic Isolation

There was a limited but significant effect of mating type on developmental failure rate (F(df = 17) = 11.33, P < 0.0001). This effect is entirely due to the higher failure rate of offspring from males of one interstrain cross involving *D*. *pseudoobscura* USA (see Fig. 4). The simplest explanation for this result is that there is an X-linked allele in high frequency in strain 4 that reduces hatching success when in the strain 6 background. Another possibility is that strain 4 has an X-linked deleterious mutant that reduces hatching success. We discuss why we favor the first explanation and describe its consequences below.

DISCUSSION

We confirmed that there is significant premating isolation in crosses involving *D. pseudoobscura* and *D. persimilis*. However, based on our measures, PPI and early postzygotic isolation were weak. This indicates that if the evolution of premating isolation was adaptive, it was most likely driven by previously documented hybrid male sterility (Dobzhansky 1936; Prakash 1972; Orr 1987).

Premating isolation (in the form of mating failures) was stronger in mating trials involving sympatric as compared to allopatric species, as is predicted when reinforcement has been acting. Our results strengthen the case for classical reinforcement in these species (Noor 1995) by eliminating PPI as a force for adaptive evolution of premating isolation.

The asymmetry that we demonstrated in premating isola-

tion (between Dpe-M \times USA-F and USA-M \times Dpe-F) is common across a wide range of taxa (see review in Coyne and Orr 2004, pp. 226-227). Such asymmetries can potentially provide further evidence for reinforcement. In our study, matings between D. pseudoobscura USA females and D. persimilis males were much less likely than matings in the opposite pairing, between D. persimilis females and D. pseudoobscura USA males. This could either be because D. pseudoobscura USA females are more discriminating or because D. persimilis males court less vigorously. Female discrimination seems the more likely explanation because males are known to court females from distant populations as vigorously as their own (Noor 1995, 1996). If differences in female discrimination are responsible for this asymmetry, the reason may be that the lines used here have different levels of experience with the other species and therefore different selection histories. As Noor (1995) points out, the historical pattern of interspecific encounter rates can influence the probability of matings between species. We were unable to test this possibility with our data because we crossed lines with very different historical likelihoods of encountering heterospecifics. For example, to start the experiment, we crossed D. persimilis strain 1, which is from a population where D. pseudoobscura are very common, with strain 2, which is from an area where D. pseudoobscura will rarely be encountered as a potential mate (M. Noor, pers. com. 2004). Repeating this study with careful attention to differences in selection history would be worthwhile. If selection history explains the demonstrated asymmetry in premating isolation, this would provide further evidence for reinforcement.

Despite many mechanisms that can potentially cause it, PPI does not seem to play an important role in the evolution of premating isolation in this system. Our measure of PPI was small ($\sim 2\%$ failure rate) and was no stronger in older

(D. pseudoobscura vs. D. persimilis) than more recently derived (D. pseudoobscura USA vs. Bogotá) population pairs. Our measure of PPI was also not stronger in sympatric than allopatric species pairs. It is possible that we missed some PPI by not measuring longevity effects. However, matings between D. pseudoobscura USA and Bogotá flies does not reduce longevity relative to matings within subspecies (Servedio, unpubl. data). Dobzhansky (1947) showed that fertility declines more quickly when females are mated to heterospecific males than when they mate with conspecifics. He attributed this to lower numbers of sperm being transferred in heterospecific as compared to conspecific matings. This is certainly a form of PPI, however, it will only be important when females mate infrequently (less than once every five days) and when conspecific sperm precedence is not important. Females in these species tend to mate every one to two days in nature (Markow 1996), and conspecific sperm precedence occurs (Dixon et al. 2003). Combining all the evidence for PPI in these species, PPI does not appear to be capable of contributing to reinforcement.

Although we did not find PPI to cause a strong selective effect against heterospecific mating in our study, there are certain cases in which such a result would be expected. Within *Drosophila*, strong PPI may occur in species that have an insemination reaction. In this reaction a gelatinous mass is formed in the female's reproductive tract upon mating; the mass disappears shortly in intraspecific crosses but may remain longer, lowering or eliminating fertility, in interspecific crosses. The insemination reaction is common, for example, in desert *Drosophila* (Patterson and Stone 1952). In general the likelihood of PPI causing significant selection against hybridization may depend on the biology of specific species pairs (see Servedio 2001, for instances of potentially strong PPI).

There was significant but weak early postzygotic isolation of the same order of magnitude as PPI (~7% of fertilized eggs fail to hatch). This source of isolation, which appears within as well as between populations of D. pseudoobscura USA, bears further discussion. It may be an epistatic postzygotic incompatibility, similar to those cataloged in D. simulans (Coyne et al. 1998; Presgraves 2003). This potential Dobzhansky-Muller incompatibility, which has relatively weak effects, appears to involve one locus on the X chromosome on strain 4, interacting with autosomal loci of mates from other strains. Males that received their X from strain 4 females (see "A" interstrain cross direction and "USA-M" in panel labels in Fig. 4) have offspring with reduced hatching success, regardless of whether they are involved in a conspecific or heterospecific cross, whereas males that received their X from strain 6 females are unaffected (see "B" interstrain cross direction and "USA-F" in panel labels in Fig. 4). Another possibility is that strain 4 has an X-linked deleterious mutant that reduces hatching success. This possibility is less likely because we see no obvious fitness reduction in the pure strain 4 flies. We also see no significant increased failure rate in offspring from crosses involving males that received their X from strain 6 females, which we would expect if there was a deleterious mutation on the strain 4 X that killed carrier male zygotes.

If the effect is due to an interaction between the strain 4

X and strain 6 background, this is an example of a Dobzhansky-Muller incompatibility, between populations within the subspecies *D. pseudoobscura* USA, that leads to "interpopulation" hybrid breakdown (Dobzhansky 1936; Orr 1987). As such, it would have the potential to combine with other incompatibilities to drive the evolution of premating isolation and ultimately speciation. Because strains 4 and 6 are closely related, such an incompatibility could represent a source of selection for premating isolation that would be effective even early in the speciation process, potentially driving divergence between populations within species. However, it is unlikely that this incompatibility contributed substantially to the speciation of *D. pseudoobscura* and *D. persimilis* given the stronger force of hybrid male sterility between this species pair.

When combined with the evidence for hybrid male sterility (Dobzhansky 1936; Prakash 1972) and F_2 hybrid breakdown (Orr 1987), our results support the conclusion that postzy-gotic isolation has been important while PPI has been unimportant in producing the strong premating isolation seen between *D. pseudoobscura* and *D. persimilis*.

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LITERATURE CITED

- Barton, N. H., and M. Turelli. 1991. Natural and sexual selection on many loci. Genetics 127:229–255.
- Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, MA.
- Coyne, J. A., S. Simeonidis, and P. Rooney. 1998. Relative paucity of genes causing inviability in hybrids between *Drosophila me*lanogaster and *D. simulans*. Genetics 150:1091–1103.
- Dixon, S. M., J. A. Coyne, and M. A. F. Noor. 2003. The evolution of conspecific sperm precedence in *Drosophila*. Mol. Ecol. 127: 1179–1184.
- Dobzhansky, T. 1936. Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. Genetics 21:113–135.
- ——. 1940. Speciation as a stage in evolutionary divergence. Am. Nat. 74:312–321.
- ———. 1947. Effectiveness of intraspecific and interspecific matings in *Drosophila pseudoobscura* and *Drosophila persimilis*. Am. Nat. 81:66–72.
- ——. 1973. Is there gene exchange between *Drosophila pseudoobscura* and *Drosophila persimilis* in their natural habitats? Am. Nat. 107:312–314.
- Howard, D. J., and P. G. Gregory. 1993. Post-insemination signalling systems and reinforcement. Proc. R. Soc. Lond. B Biol. Sci. 340:231–236.
- Kirkpatrick, M., and V. Revigné. 2002. Speciation by natural and sexual selection: models and experiments. Am. Nat. 159 Supplement:S22–S35.
- Knowles, L. L., B. B. Hernandez, and T. A. Markow. 2004. Exploring the consequences of postmating-prezygotic interactions

between the sexes. Proc. R. Soc. Lond. B Biol. Sci. 271 Supplement:S357–S359.

- Markow, T. A. 1996. Evolution of *Drosophila* mating systems. Pp. 73–106 *in* M. K. Hecht, R. J. MacIntyre, and M. T. Clegg, eds. Evolutionary biology. Plenum Press, New York.
- ——. 1997. Assortative fertilization in *Drosophila*. Proc. Natl. Acad. Sci. USA 94:7756–7760.
- Marshall, J. L., M. L. Arnold, and D. J. Howard. 2002. Reinforcement: the road not taken. Trends Ecol. Evol. 17:558–563.
- Mayr, E. 1946. Experiments on sexual isolation in *Drosophila*. VII. The nature of the isolating mechanisms between *Drosophila pseudoobscura* and *Drosophila persimilis*. Proc. Natl. Acad. Sci. USA 32:128–237.
- Noor, M. A. F. 1995. Speciation driven by natural selection in *Drosophila*. Nature 375:674–675.
- . 1996. Absence of species discrimination in *Drosophila pseudoobscura* and *D. persimilis* males. Anim. Behav. 52: 1205–1210.
- Orr, H. A. 1987. Genetics of male and female sterility in hybrids

of *Drosophila pseudoobscura* and *D. persimilis*. Genetics 116: 555–563.

- Patterson, J. T., and W. S. Stone. 1952. Evolution in the genus Drosophila. Macmillan, New York.
- Powell, J. R. 1983. Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: evidence from *Drosophila*. Proc. Natl. Acad. Sci. USA 80:492–495.
- Prakash, S. 1972. Origin of reproductive isolation in the absence of apparent genic differentiation in a geographic isolate of *Dro*sophila pseudoobscura. Genetics 72:143–155.
- Presgraves, D. C. 2003. A fine-scale genetic analysis of hybrid incompatibilities in *Drosophila*. Genetics 163:955–972.
- Price, C. S. C., C. H. Kim, C. J. Gronlund, and J. A. Coyne. 2001. Cryptic reproductive isolation in the *Drosophila simulans* species complex. Evolution 55:81–92.
- Servedio, M. R. 2001. Beyond reinforcement: the evolution of premating isolation by direct selection on preferences and postmating, prezygotic incompatibilities. Evolution 55:1909–1920.

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