Relative mating success of hybrid and pure species males to highly discriminating *D. pseudoobscura* females.

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Introduction

In nature, it is often advantageous to mate preferentially within one's species because of negative consequences associated with heterospecific mating. This discrimination against heterospecifics is often driven by female choice rather than male choice due to the large investment of females into small numbers of eggs relative to that of males into large numbers of sperm. Thus, female eggs are wasted on heterospecific matings when the resultant offspring are unfit, and her reproductive fitness is significantly decreased. In contrast, in many species, males can waste many sperm without a significant cost to their potential reproductive output. In the North American species *Drosophila pseudoobscura* and *D. persimilis*, the cost of interspecies mating is that F_1 male progeny are sterile; therefore, females have a great incentive to discriminate against mating with heterospecific males.

The extent to which females discriminate against heterospecifics is related to their proximity to other species (Howard, 1993; Noor, 1999; Servedio and Noor, 2003). Reinforcement is the process whereby mate discrimination is strengthened via natural selection to prevent maladaptive hybridization. As a result, while species with geographically overlapping ranges may need high mating discrimination, those with disjunct ranges can be less discriminating. *D. pseudoobscura* females from Mather, California, are hypothesized to be highly discriminating, because they co-occur with *D. persimilis* (Noor, 1995; Noor and Ortiz-Barrientos, 2006), and indiscriminate mating by the females could result in wasteful interspecific matings, because they produce sterile male offspring.

Identifying the phenotypic cues associated with species discrimination can help us understand how it is mediated in nature. Mate discrimination by *D. pseudoobscura* females is thought to originate from two sources: male courtship song parameters and olfactory cues (Ortiz-Barrientos *et al.*, 2004). Male courtship songs are thought to be responsible for some basal mate discrimination in these species (Williams *et al.*, 2001), and olfactory cues (*e.g.*, cuticular hydrocarbons) may be responsible for the enhanced discrimination used in reinforcement (Ortiz-Barrientos *et al.*, 2004). Thus, if true, in highly discriminating Mather females, both male courtship songs and cuticular hydrocarbons would be involved in recognition of conspecific mates.

The hypothesis above predicts that allopatric Flagstaff *D. pseudoobscura* strains use only male courtship song to discriminate conspecifics from heterospecifics. In support of this hypothesis, F_1 males (Flagstaff males × *D. persimilis* females) mate with Flagstaff females as well as do pure-species *D. pseudoobscura* males, despite the F_1 males bearing a cuticular hydrocarbon profile similar to *D. persimilis* (Noor and Coyne, 1996). A comparable mating study of the highly discriminating *D. pseudoobscura* Mather 17 strain and its F_1 hybrids with *D. persimilis* was not conducted.

The F_1 progeny of *D. pseudoobscura* Mather 17 females and *D. persimilis* males have a cuticular hydrocarbon similar to *D. persimilis* (Noor and Coyne, 1996). If Mather 17 females use both male courtship songs and cuticular hydrocarbons to distinguish between conspecifics and heterospecifics, they may mate less frequently and have longer copulation latencies with F_1 males than with conspecific Mather 17 males, because the F_1 males will seem to have olfactory cues similar

to *D. persimilis*. Mather 17 females should also be still more discriminating against mating with *D. persimilis* males, and thus these males should have the lowest mating frequency and the longest copulation latencies. Hence, for matings with *D. pseudoobscura* Mather 17 males, we predict the success to be Mather 17 males > F_1 males > *D. persimilis* males.

Methods

Mating Trials:

Drosophila pseudoobscura Mather 17 (Mather 17) virgin females were mated with *D. pseudoobscura* Mather 17 males, F_1 (Mather 17 females × *D. persimilis* Mount St. Helena 1993 males) males, and *D. persimilis* Mount St. Helena 1993 (MSH 1993) males each day possible from January to April 2007. Matings were conducted in the morning between 9:00am and 10:30am, coinciding with "lights-on" in the incubator's 12-hour light-dark cycle. Approximately equal numbers of flies of each type were assayed each day, hence reducing artifacts of day-to-day variation in environmental factors.

For each mating that was conducted, an eight-day post-eclosion Mather 17 virgin female was placed into a food vial with one virgin male, which could be Mather 17, F_1, or MSH 1993 male, and a cotton plug was pushed down until the flies had only ½ inch of open space. This ensured that the flies came into contact with one another. For each mating trial, the start time, the time of courtship, and the time of copulation were recorded using the timer, and the courtship and copulation latencies (in seconds) were calculated using these data. If the male fly did not court or mate, that was recorded. Courtship was indicated by the males' wing vibrations toward the female, and the pair was only considered to be mating if the copulation lasted more than thirty seconds (though the initiation of the copulation was recorded as the mating time in the data). If the male fly had not courted ten minutes after being put into the vial with the female, the pairing was recorded as "no courtship". If the male fly had not mated ten minutes after the first bout of courting the female, it was recorded as “no copulation”.

Statistical Analysis:

The results were analyzed via Chi Square test and Mann-Whitney U-tests. The Chi Square test was used for categorical data, such as matings (Y/N) to particular types of males (Mather 17 vs. F_1 vs *D. persimilis*). The non-parametric Mann-Whitney U-tests were used for continuous data, such as the length of time it took for each type of male to either recognize and court (courtship latency) or succeed in copulating with (copulation latency) a female.

Results

There was a significant difference between the number of *D. persimilis* MSH 1993 males that mated with *D. pseudoobscura* Mather 17 virgin females and the number of Mather 17/F_1 males that mated with Mather 17 virgin females (Chi Square P-Value < 0.0001: Table 1). Thus, as reported previously, Mather 17 females are highly discriminating against heterospecific males.

However, contrary to our prediction, there was no significant difference between the number of Mather 17 and F_1 males that mated with Mather 17 virgin females after courting (Chi Square P-Value = 0.4224: Table 2). Mather 17 males mated 47 times and did not mate 10 times after courting, and F_1 males mated 44 times and did not mate 6 times (Table 2). There was also no significant difference in the copulation latency between Mather 17 and F_1 males using the Mann-Whitney U test (P-value = 0.0728) or mean copulation latency in seconds (Tables 3 and 4). Indeed, the opposite trend was observed: F_1 males mated slightly more and slightly faster.

Table 1. The number of *D. pseudoobscura* Mather 17, F_1, and *D. persimilis* MSH 1993 males that did and did not mate Mather 17 virgin females. Only those males that courted the females were included in the tally. (Ch Square P-Value < 0.0001 for Mather 17/F_1 males vs MSH 1993 males).

Mated? (Y/N)	Mather 17 Males	F_1 Males	MSH 1993 Males
N	10	6	31
Y	47	44	6
Totals	57	50	37

Table 2. The number of Mather 17 and F_1 males that did and did not mate Mather 17 virgin females. Only those males that courted the females were included in the tally. (Chi Square P-Value = 0.4224).

Mated? (Y/N)	Mather 17 Male	F_1 Male
N	10	6
Y	47	44
Totals	57	50

Table 3. Mann-Whitney U information for copulation latency of Mather 17 and F_1 males to Mather 17 virgin females. Only those males that courted the females were included in the tally. (P-Value = 0.0728).

Male Type	Count	Sum Ranks	Mean Rank
Mather 17	56	3279.5	58.6
F_1	50	2391.5	47.8

Table 4. The mean copulation latency, in seconds, for those Mather 17 and F_1 males that both courted and mated Mather 17 virgin females.

Male Type	Count	Mean (s)	Std. Dev.	Std. Err
Mather 17	47	78.7	118	17.2
F_1	44	46.7	102	15.4

Table 5. Mann-Whitney U information for courtship latency of Mather 17 and F_1 males to Mather 17 virgin females. (P-Value = 0.0044).

Male Type	Count	Sum Ranks	Mean Rank
Mather 17	58	2788	48.1
F_1	54	3540	65.6

Table 6. The mean courtship latency, in seconds, for Mather 17 and F_1 males that courted Mather 17 virgin females.

Male Type	Count	Mean (s)	Std. Dev.	Std. Err
Mather 17	56	41.2	58.9	7.9
F_1	50	81.5	111.9	15.8

This pattern could have resulted from an experimental artifact associated with inbreeding: despite being sterile, F_1 males may be slightly more vigorous than Mather 17 males and were thus able to secure more copulations. However, contrary to this hypothesis, F_1 males were significantly slower to court *D. pseudoobscura* females than were Mather 17 males (Tables 5 and 6).

Discussion

As predicted, *D. persimilis* MSH 1993 males are very unsuccessful at mating with *D. pseudoobscura* Mather 17 females. Given that males of these species do not exhibit any species discrimination (Noor, 1996; Kandul *et al.*, 2006), this finding almost certainly results in large part from the high level of species mate discrimination by Mather 17 females.

However, contrary to our prediction, Mather 17 females are no more discriminating against F_1 males relative to Mather 17 males. Indeed, the opposite was true: F_1 males were slightly (but not significantly) more successful at mating with Mather 17 females than were Mather 17 males. We further failed to find evidence that this pattern was an artifact of inbreeding.

Ortiz-Barrientos *et al*. (2004) suggested that these females used olfaction as part of their species discrimination, because they identified several olfaction-related genes in the region of the genome to which a mate preference QTL mapped. At least three explanations can reconcile the inconsistency of this suggestion with our findings. One possibility is that the hypothesis of Ortiz-Barrientos *et al*. (2004) was incorrect, and highly discriminating *D. pseudoobscura* females are not necessarily using olfactory cues. Alternatively, *D. pseudoobscura* females may use olfactory cues, but they are prioritized below auditory cues such as courtship song. F_1 males from the crosses we conducted would have courtship songs very similar to *D. pseudoobscura* (Noor and Aquadro, 1998), and perhaps these females needed the combination of song and olfactory cue to discriminate. Finally, olfactory cues besides the cuticular hydrocarbons studied previously may mediate species discrimination in this population.

This experimental design does not mimic natural environmental conditions, and there is a concern with overinterpreting such results. It would also be useful to control for inbreeding depression that may have existed in the Mather 17 or MSH 1993 stocks by using flies one generation removed from nature. Further, detailed observations of the process of courtship itself, as done by some classic studies in these species (Brown, 1964; Brown, 1965), may identify specific points at which courtship breaks down in interspecies pairings.

Conclusion

D. pseudoobscura Mather 17 females do not show any discrimination towards F_1 males in terms of mating frequency or copulation latency, potentially in contrast to the prediction that cuticular hydrocarbons are responsible for secondary reinforcement-based discrimination in *D. pseudoobscura* females. Further studies should be conducted to confirm the results reported here.

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Tolerance adaptation of *Drosophila melanogaster* to increased salt concentration due to new beneficial mutations.

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Abstract

Mutation and selection are major evolutionary forces that help organisms to adapt to novel environments. Although numerous laboratory experiments with model organisms and observations from nature have demonstrated adaptations to new environmental conditions, it is difficult to distinguish whether adaptation has occurred due to new beneficial mutation or by selection on preexisting genetic variation. By using a highly inbred homozygous stock we have demonstrated adaptation of *Drosophila melanogaster* to increased tolerance to previous toxic levels of dietary salt as a result of new beneficial mutations. The adaptation occurred quickly (seven generations) suggesting that a small number of genes might have been involved and that new mutations can play an important role in adaptive evolution.

Introduction

Mutation and selection are important interactive forces responsible for major evolutionary changes in all organisms. Yet, whether evolution is driven by natural selection acting on new mutations or on preexisting genetic variation is an ongoing debate (Nei, 1987; Lande, 1988; Gillespie, 1991; Li, 1997; Lynch, 1996; Barton, 1998; Orr and Betancourt, 2000; Kim and Stephan, 2002; Orr, 2005). There are numerous examples of rapid adaptations in natural populations, including insecticide resistance, adaptive melanism in populations of rock pocket mice, pelvic armor loss in fresh water sticklebacks, evolution of speech in humans, metal tolerance in plants, and HIV resistance in humans, that may be caused by new advantageous mutations (Wood and Bishop, 1981; Macnair, 1993; Stephens *et al.,* 1998; Toma *et al.*, 2002; Daborn *et al*., 2002; Nachman and Hoekstra, 2003; Shapiro *et al*., 2004). It is difficult, however, to disentangle the effects of new mutations from preexisting genetic variation. A number of studies have shown that adaptations can occur quickly due to preexisting genetic variation (Moya *et al.,* 2005; Peichel, 2005; Hartley *et al*., 2006; Zhan *et al*., 2006). New beneficial mutations have been studied experimentally in lines of microorganisms, including adaptation of clones of *Escherichia coli* to high and low temperatures (Bennett *et al*., 1992) and yeast to a low phosphate chemostat environment (Francis and Hansche, 1972). Few experiments, however, have been performed with multicellular organisms to measure directly the influence of new beneficial mutations on fitness and adaptation (Francis and Hansche, 1972; Batallion, 2000).

Drosophila is also a widely used model system to study adaptations to new environmental conditions such as new food source, temperature fluctuations, osmotic stress, hypoxia, and starvation