

Notes and Comments

Forced Matings in Natural Populations of *Drosophila*

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Forced mating has been reported for a number of vertebrate and invertebrate taxa. The implications of forced copulation for mating system evolution, however, has received the greatest consideration for birds (Gowaty and Buschhaus 1998). Insect species in which females mate before full maturation represent a different slant on forced mating. Some lepidoptera, for example, exhibit the unusual phenomenon of pupal mating, in which males are attracted to unclosed females and mate with them before their emergence (Brower 1997). The degree to which females in pupal-mating species exercise mate choice is questionable.

Species of the genus *Drosophila*, especially *Drosophila melanogaster*, provide one of the most popular model systems for the study of sexual selection and sexual isolation. Studies of reproductive behavior in *Drosophila* have commonly assumed that females control whether a mating will take place and with whom. Exceptions include conditions of artificially high density, such as in laboratory population cages (Burnet and Connolly 1974), in which females, unlike under natural conditions, cannot escape the advances of courting males. The assumption of female control is reflected in the names of the experimental paradigms employed, such as the "female choice" design and the "no choice" design. Laboratory studies always employ sexually mature flies that are several days old. While the merits of one design over another depend upon the question being addressed, all have two common assumptions. The first assumption is that under the conditions of the tests, flies

of neither sex are forced to mate. Second, the results are assumed to have relevance to the natural history of the species involved, making the conclusions useful in elucidating general evolutionary patterns and processes. Unfortunately, the natural history of reproductive behavior in *D. melanogaster* is less well known than for endemic *Drosophila* such as the Hawaiian species (Kambysellis and Craddock 1991).

Male *Drosophila* must perform species-specific courtship behaviors, even if these are minimal (e.g., copulation attempts), before mating will occur (Spieth 1952). Species of *Drosophila* differ widely with respect to the age at which males will court receptive females (Markow 1996). Sexual maturity in female *Drosophila*, the age at which females are receptive to courting males, also varies between species (Markow 1996). While sexually mature female *Drosophila* employ a range of behaviors to thwart unwanted advances of courting males, such as decamping, wing flicking, kicking, and ovipositor extrusion (Speith 1952), newly emerged (teneral) females do not perform any of these rejection behaviors (Manning 1967; Connolly and Cook 1978). The cuticles of teneral flies have not yet hardened, their wings are folded, and they move slowly. Investigators are generally aware of the ages at which males and females of various *Drosophila* species become sexually mature and avoid using immature flies in any of the experimental designs.

To place laboratory studies of sexual selection in a natural context, several investigations of *Drosophila* courtship and copulatory success have been conducted in the field (Partridge et al. 1987; Gromko and Markow 1993; Markow et al. 1996). In two of these (Partridge et al. 1987; Gromko and Markow 1993), the authors noted the low numbers of copulations encountered given the large numbers of flies being observed and the high frequency of courtship. Yet examination of field-caught females of *D. melanogaster* and *Drosophila simulans* reveals that nearly 100% have been inseminated (Bouletreau 1978; Gromko and Markow 1993).

In nature, *D. melanogaster* and *D. simulans* exhibit peaks in mating activity during the morning and evening (Grom-

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ko and Markow 1993). On one morning in April 1990, I was collecting copulating pairs on fallen citrus in Tempe, Arizona, until the time when matings became infrequent. While searching among pieces of fallen fruit, I noticed what at first I thought was a teneral fly being attacked by an ant. Closer inspection revealed that it was not an ant but a mature *D. melanogaster* or *D. simulans* male and that he was copulating with a virgin female so recently emerged from the pupa case that her body was still a transparent white and her wings were still folded. Looking around in the leaf litter and dried out pieces of fruit from which emergence was occurring, I found several more copulating pairs that involved mature males and teneral females.

These observations raised several questions subsequently addressed with additional field observations and laboratory experiments: What species were involved in matings of newly emerged females? How long do the copulations last? Are offspring produced by the matings?

Emergence sites in fallen citrus in Tempe, Arizona, were searched for mating pairs in which the female was teneral (white cuticle, folded wings). When a pair was located, the time was recorded and the pair was left undisturbed until copulation ended. The time at the end of copulation was noted, and the two flies were aspirated immediately into separate vials and brought to the laboratory for identification. The species of the male, *D. melanogaster* or *D. simulans*, was noted and the female was transferred every 3 d for 12 d to a fresh vial with live yeast. Vials were retained to score the numbers of progeny from each female.

Results of two different field collections are shown in table 1. Data from the first observation period involved 18 h of observations over 9 d, while the second study represents 22 h over 11 d. Because females of the two species are too similar to distinguish, only the males could

be confidently identified as to species. In the first study, 23 of the 41 teneral females seen in the leaf litter were *in copula*. In the second, 33 of 54 females were mating. The majority of the matings involved *D. simulans* males in both periods, consistent with the proportions of *D. simulans* and *D. melanogaster* at the sites. Not all matings involving *D. melanogaster* or *D. simulans* males produced progeny. Of those that did produce progeny, there was an average of about 100 offspring per female. The number of offspring produced was significantly lower in matings involving male *D. simulans* in 1990 but not 1997. The copulation durations are actually underestimates, as no initiations of copulation were actually observed in the field.

Drosophila melanogaster and *D. simulans* are sibling species in which males may be distinguished by their external genitalia, but females cannot be reliably separated. Because it was too difficult to know the species identity of females in infertile matings, and thus to assess whether interspecific copulations occur, additional studies were conducted in the laboratory.

Uncrowded cultures of *D. melanogaster* and *D. simulans*, founded three generations earlier from multifemale collections at Tempe, were used to collect virgin males and females. Flies were separated with aspirators (no anesthesia). Males were stored five per yeasted vial until 8 d of age. Females were collected with aspirators directly upon emergence and immediately transferred to vials containing two 8-d-old males. The two males were either both conspecific or both heterospecific with respect to the female. Vials were observed closely for 1 h (the teneral period lasts 2 h) and discarded if no copulation took place. When a mating was observed, the extra male was removed, and the copulation duration was recorded. After the pair disjoined, the female was saved and transferred every 3 d for 12 d to a fresh vial to assess her productivity. When placed with newly emerged females, laboratory males of both *D.*

Table 1: Field and laboratory observations on the copulations, their durations, and productivity between mature adult males and newly emerged females of *Drosophila simulans* and *Drosophila melanogaster*

Condition and species	No. of pairs tested	No. of matings observed	Copulation duration (min \pm SE)	% fertile matings (<i>n</i>)	Mean no. of progeny/fertile mating (\pm SE)
Field, 1990:					
<i>Drosophila simulans</i> ♂	NA	15	39.7 \pm 3.4	67 (10)	79.5 \pm 10.6
<i>Drosophila melanogaster</i> ♂	NA	8	37.0 \pm 2.6	62 (5)	120.0 \pm 16.8
Field, 1997:					
<i>D. simulans</i> ♂	NA	26	34.1 \pm 2.9	62 (16)	101.9 \pm 8.2
<i>D. melanogaster</i> ♂	NA	7	40.3 \pm 5.6	71 (5)	98.2 \pm 7.3
Laboratory:					
<i>D. simulans</i> ♀ \times <i>D. simulans</i> ♂	79	34	16.6 \pm 1.1	24 (8)	103.7 \pm 9.9
<i>D. simulans</i> ♀ \times <i>D. melanogaster</i> ♂	17	3	14.0 \pm 5.2	0	0
<i>D. melanogaster</i> ♀ \times <i>D. melanogaster</i> ♂	56	24	12.1 \pm 2.5	79 (19)	120.8 \pm 13.5
<i>D. melanogaster</i> ♀ \times <i>D. simulans</i> ♂	28	10	15.7 \pm 6.1	0	0

simulans and *D. melanogaster* were observed to copulate (table 1). In more than half of the cases, copulation occurred in the absence of courtship. When courtship occurred, it lasted <10 s. Males that did not copulate usually had not courted. A low number of interspecific copulations between *D. simulans* and *D. melanogaster* were also observed, but none produced progeny. In no case was a teneral female observed to exhibit any form of rejection behavior.

Important differences were found between the outcomes of laboratory and field matings with teneral females. Copulations were two to three times as long in duration in the field, an observation that is also true of matings with mature females (Gromko and Markow 1993). Only one-quarter of *D. simulans* teneral females mated in the laboratory produced progeny. Laboratory-mated teneral females that did produce offspring were as productive as field-mated teneral females. A number of laboratory-mated females of both species held their ovipositors to one side after mating, suggesting they had been damaged by mating. This condition was not observed in field-mated females.

There may be an undetected difference in the way these matings occur in nature. Teneral females may be copulating closer to the time of emergence in nature, especially if males are patrolling emergence sites. In the laboratory, on the other hand, I moved teneral females by aspiration to vials with waiting males. Another potential explanation is that males in nature are a different age or mating status than laboratory males. Approximately 30% of field matings did not produce progeny. It is too difficult to know if this was because some of the field matings were interspecific. In the laboratory, none of the interspecific matings were fertile. Other potential explanations are that fewer sperm are transferred in teneral copulations or that sperm are either destroyed or unutilized (Gowaty 1997; Gowaty and Buschhaus 1997).

Because males of both *D. simulans* and *D. melanogaster* subsequently were found to engage in forced mating, another species, *Drosophila mojavensis*, which has a very different mating system, was also examined in the laboratory. Mated females of *D. melanogaster* and *D. simulans* typically do not remate for 4 or 5 d (Markow 1996). In these species, males typically attempt, unsuccessfully, to copulate with unreceptive mature females despite vigorous rejection signals (Markow and Hanson 1981). In many other *Drosophila* species, such as *Drosophila mojavensis*, females remate daily (Markow 1982). Males of this species never attempt copulation unless females have performed a stereotypical acceptance signal (Markow and Toolson 1990), predicting that forced matings would not be observed.

The same testing procedure was followed for *D. mojavensis*, except that males used in experiments were 12 d

old because males of this species require longer to mature than males of the other two species (Markow 1982). All tests of *D. mojavensis* were between conspecific males and females. The stock of *D. mojavensis* was derived five generations previously from a multifemale collection from their host plant, organ pipe cactus (*Stenocereus thurberi*) at San Carlos, Sonora, Mexico, in May 1990. No copulations of teneral females were seen in 108 vials.

The absence of teneral matings in *D. mojavensis* suggests that the behavior of *D. melanogaster* and *D. simulans* males may reflect the comparatively low number of mating opportunities in nature for males of these two species. In addition to the pattern of daily remating in *D. mojavensis*, males require several days longer than females to become sexually mature, while males and females of the other two species mature at similar ages. These factors produce a more male-biased operational sex ratio in *D. melanogaster* and *D. simulans*, and the more intense competition for mates may favor tactics such as the patrolling of emergence sites, in order to copulate with females incapable of rejecting them (Gowaty 1997).

Flies of the genus *Drosophila* have been popular organisms for laboratory investigations of sexual behavior and its implications for fitness. The present observations indicate typical laboratory-mating experiments may be measuring only part of what might be occurring in nature. Laboratory studies, unless specifically examining remating, universally employ virgin females that have been aged several days. In nature, however, virgin females of either *D. melanogaster* or *D. simulans*, if they mate when newly emerged, do not exercise choice to the extent assumed by laboratory studies. Matings with teneral females may, in fact, be forced matings, as females appear incapable of performing rejection behaviors. Indeed, female receptivity depends upon maturation of the copora allatum (Manning 1967), supporting the interpretation that teneral females are being forced to mate. Field studies of *Drosophila* mating may, in many instances, actually be observations of rematings by the females. Finally, productivity of matings by sexually mature *D. melanogaster* have been reported to be from 150 (Markow et al. 1978) to over 400 (Gilbert et al. 1981) progeny, considerably higher than observed for teneral matings in either the field or the laboratory.

The extent to which first matings in nature occur when females are teneral is unknown and may depend upon factors like population density and age structure. Male *D. melanogaster* also have been observed to seek emerging females at field sites in Australia (A. Hoffmann, personal communication). If teneral mating in nature is as common as it appears from this study in *D. melanogaster* and *D. simulans*, it calls for a reinterpretation of the evolutionary implications of laboratory experiments for the natural reproductive biology of these species. Specifically, for *D. me-*

lanogaster and *D. simulans*, the role of female choice in intra- and intersexual selection in nature may not be what is assumed from laboratory studies of mating success. If the potential for mate choice is restricted, not only does it raise questions as to the robustness of the role of choice in the mating behavior of females of these species, but it suggests that the potential for the evolution of post-copulatory mechanisms of fertilization control be more closely evaluated. Furthermore, if copulation is toxic to females, as suggested by (Chapman et al. 1995), and if, in nature, force-mated females mate earlier than females in laboratory experiments, the costs to females of mating, and thus the intensity of selection on postcopulatory processes (Gowaty 1997; Gowaty and Buschhaus 1997), may be greater than originally assumed (Rice 1996).

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