

Male size, developmental stability, and mating success in natural populations of three *Drosophila* species

THERESE ANN MARKOW & JEFFRY P. RICKER

Department of Zoology, Arizona State University, Tempe, AZ 85287-1501, U.S.A.

Morphological correlates of male mating success were assessed in natural populations of three *Drosophila* species. Mating males in *D. simulans* were larger than single males but were characterized by reduced developmental stability as indicated by fluctuating asymmetry. Mating male *D. pseudoobscura* were no larger than single males but exhibited significantly greater developmental stability. In *D. mojavensis*, however, mating males were larger and they showed a similar level of fluctuating asymmetry to that observed in single males. The differences observed between species are discussed in the context of their mating systems and reproductive ecology.

Keywords: *Drosophila*, fluctuating asymmetry, sexual selection.

Introduction

Male mating success in *Drosophila* has been extensively studied in the laboratory and appears to be related to male size for a number of species (Partridge *et al.*, 1987a; Markow, 1985; Markow, 1987b; Markow, 1988). Very few studies of *Drosophila* male mating success have been conducted in the field but those which have been reported also support the importance of male size in *D. melanogaster* (Partridge *et al.*, 1987a; Markow, 1988), *D. nigrospiracula* (Markow, 1988), *D. buzzatii* (Santos *et al.*, 1988) and *D. testacea* (James & Jaenike, 1991). The relationship is less consistent for *D. pseudoobscura* observed at baits in that not all replicates showed an advantage for large males (Partridge *et al.*, 1987b), which suggests that in some species directional selection for male size may not always be associated with mating success.

A number of investigations report a reduction in fitness of individuals whose phenotypes lie towards the ends of distributions, supporting the action of balancing or stabilizing selection (Lerner, 1954; 1958). The extent to which balancing sexual selection occurs in natural *Drosophila* populations has not been examined. However, body size is a quantitative trait, and individuals much larger or smaller than the population average are expected to show reduced developmental homeostasis due to homozygosity at the loci contributing for their large or small phenotypes. Thus the prediction follows that if successfully mating males are signifi-

cantly larger, they should also tend to exhibit less developmental stability. A contrasting prediction is that if there is balancing sexual selection on male size, it should be the developmentally stable, average size males who experience the greatest success as these males are closest to the population average.

Developmental stability has been measured in *Drosophila* but never as it relates to mating success. The most widely employed measure of developmental homeostasis is fluctuating asymmetry, or the difference in magnitude of a trait between the right and left side (Palmer & Strobeck 1986). The bilateral traits most often used to calculate fluctuating asymmetry in *Drosophila* have been numbers of bristles, especially the sternopleurals (Reeve, 1960; Van Valen, 1962; Parsons, 1962; Markow & Ricker, 1991). However, any paired structure may show fluctuating asymmetry.

The present study sought to accomplish several things. First, we wished to assess potential correlates, in addition to male size, of mating success in order to gain a more complete understanding of sexual selection in nature. Second, we wished to expand the existing database on mating behaviour in natural *Drosophila* populations to include additional species. In the present study, we asked if mating males could be distinguished from non-mating males by their size or developmental stability in several species. We calculated fluctuating asymmetry for several independent measures, wing length and regional bristle numbers, to examine the relationship between developmental

stability and mating success in three *Drosophila* species.

Three unrelated species were selected for study: *D. simulans*, *D. pseudoobscura*, and *D. mojavensis*. *Drosophila simulans* is the sibling species of *D. melanogaster* with whom it is often sympatric. In many Tempe, Arizona citrus groves, *D. simulans* are more common than *D. melanogaster*, although both may be collected from the same piece of fruit. At the same localities, *D. pseudoobscura* are found in smaller numbers throughout the winter and spring. The last species, *D. mojavensis* is endemic to the Sonoran Desert and utilizes necrotic arms of columnar cacti to feed and reproduce. These three species were selected because their mating systems are under investigation in our laboratory and because they are frequently discussed in the genetics, evolution, and behaviour literature.

Methods

Field observations

Drosophila simulans and *D. pseudoobscura* were observed on fallen citrus (lemons and grapefruit) in Tempe, Arizona. The distribution of males and females on different types of fallen citrus was assessed on three different days. Fruit types were classified as in Markow (1988) as being newly fallen and cracked open exposing the citrus flesh, fallen but with the skin intact, or decaying. *Drosophila simulans* was the predominant species, with *D. pseudoobscura* the next most common. Occasional *D. melanogaster* males were collected but discarded from the samples. Mating activity shows two peaks, one in the morning, the other just prior to dusk (Markow, 1987a). Flies were collected at both activity periods for about 1 week during May of both 1988 and 1989. As soon as a mating pair was observed, it was aspirated into a vial and a single male was immediately aspirated from the same area into another vial. Flies were taken back to the lab-

oratory each day to be measured for thorax length and for wing mounting (see below).

Drosophila mojavensis were observed on their natural host plant, organpipe cactus (*Stenocereus thurberi*) at our long-term study site in the desert near San Carlos, Sonora, Mexico (Markow & Toolson 1990). Three different observations/collection periods were employed on three different cacti in March 1989. Distribution of males and females at various locations on necrotic cacti were reported previously in Markow & Toolson (1990). As with the other two species, when a copulating pair was observed, it was aspirated into a vial and a single male from nearby was immediately aspirated into a second vial. Thorax lengths were measured in Mexico. Wings were prepared for measuring in Mexico but returned to the laboratory in Tempe.

Measurements on flies

Thorax lengths were measured under a dissecting microscope with an ocular micrometer. Several other bilateral traits were measured. Wing lengths were measured for males of both species. Wings were removed with forceps after thorax lengths were recorded and placed on double-sided transparent tape that had been affixed to a clear 35-mm projection slide. Wings were projected onto a wall covered with paper for measuring. The length measured was from the anterior crossvein to the end of the second longitudinal vein (Fig. 1) and was in arbitrary units. Although the position of the projector with respect to the wall was marked on the floor in order to be consistent in measurements between years, a seemingly imperceptible change in the position set-up in the second year caused some slight differences in the wing measurements between years. In the second year, additional traits were added to those measured for *D. simulans* and *D. pseudoobscura*. These included the number of sternopleural bristles, orbital and frontal bristles, and arisal branches (Van

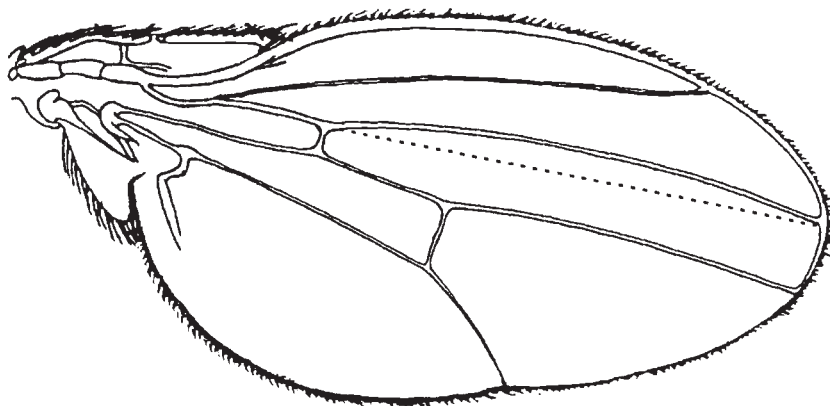


Fig. 1 Line for wing length measure extended from the anterior crossvein to the end of the second longitudinal vein.

Valen, 1962), all counted under a dissecting microscope. The dark coloration interfered with our ability to count sternopleurals or orbital bristles in *D. pseudoobscura*.

Analysis of fluctuating asymmetry (FA)

Asymmetry scores were calculated by subtracting the value of the left side from that of the right for each of the traits. Several factors are known to confound analysis of fluctuating asymmetry (Palmer & Strobeck, 1986) and must be tested for and corrected if they exist. One is the existence of a positive correlation between the magnitude of the trait and the degree of asymmetry. The other is the presence of directional asymmetry. In one species, *D. pseudoobscura*, wing length and wing length asymmetry were correlated ($r=0.46$, $P<0.01$), which was corrected for by dividing each individual ($R-L$) value by the average magnitude of the trait ($(R+L)/2$). Directional asymmetry (DA) was measured by factorial ANOVA using side as a factor. None of the traits were found to exhibit DA in any species. When the factor 'male-type' (i.e. single or mating) shows statistical significance, a group difference in FA is indicated.

Results

Drosophila simulans

Sex ratios differ dramatically on each of the three fruit types (Table 1). On the exposed flesh of fruit that had recently broken open, females significantly outnumbered males. It was easy to observe females ovipositing on this class of fruit, and within a day, the fruit surface was usually plastered with eggs. Few copulations were observed on fruits of this type. There was a slight but significant male bias on the skin of intact fruit. The slight female bias on decaying fruit was not significant. Flies were observed to be feeding on the microbial flora on the fruit in this class. There was considerable courtship activity among flies on decayed fruit. Males would court any female encountered. Most of the copulations were observed on pieces of decaying fruit, although for the total number of flies present and the level of courtship activity, copulations were rare which suggests that most females were unreceptive.

Mating and single males that were used for measurements were all collected from decaying fruit (Table 2). Males caught in copula in both years, were longer in the thorax. Wing lengths of mating males were greater only in the first year. The length of the thorax of all flies was larger in the second year but the difference in wing length between the 2 years was due to the artefact described in the methods section. Wing length and thorax length were highly correlated ($r=0.7669$, $P<0.0001$). Single and mating males did not differ in the additional quantitative characters measured in the second year: arisal branch number, head bristle number, or sternopleural bristle number. There was no relationship between wing length and wing length fluctuating asymmetry in this species ($r=0.18961$, $P=0.1872$). Fluctuating asymmetry was always greatest in the copulating males but the difference was significant only for wing length and arisal branching. The direction of wing fluctuating asymmetry differences for male type was consistent in both years. None of the measures of fluctuating asymmetry were correlated. Thus copulating males of *D. simulans* were larger and, as such, exhibited the expected increase in fluctuating asymmetry.

Drosophila pseudoobscura

Far fewer flies of *D. pseudoobscura* than of *D. simulans* were at the site. While a large number of *D. simulans* remained on fallen fruit throughout the day and night (Markow, 1987a), *D. pseudoobscura* came to the fruit for only about 1 h in the morning and again in the early evening. The distribution of males and females (Table 1) reveals that the only departure from a 1:1 sex ratio was on the decayed fruit where it was significantly male-biased. As in *D. simulans* the majority of copulations occurred on the decaying fruit. *D. pseudoobscura* of both sexes were also observed to feed on the decaying fruit but females were never observed ovipositing. The behaviour of *D. pseudoobscura* males shows some obvious differences with *D.*

Table 1 Distributions of flies collected on flesh of cracked-open fruit, skin of intact fruit, and decayed fruit. For both species, data from different days were tested for homogeneity prior to pooling

	Females		Males		$\chi^2(1:1)$
	<i>n</i>	%	<i>n</i>	%	
<i>D. simulans</i>					
Flesh	356	65.3	189	34.7	51.17
Skin	201	42.0	277	58.0	12.08
Decayed	124	54.1	105	45.9	1.58
Total	681	54.4	571	45.6	2.62
<i>D. pseudoobscura</i>					
Flesh	85	52.8	76	47.2	0.50
Skin	31	50.1	30	49.9	0.02
Decayed	44	39.3	68	60.7	5.14
Total	160	47.9	174	52.1	0.59

Table 2 Characteristics of single and mating males in a natural population of *D. simulans*

	Single male (<i>n</i> = 50)	Copulating male (<i>n</i> = 50)
1988		
Thorax length	0.8743 ± 0.01	0.9049 ± 0.01*
Wing length	48.89 ± 0.71	51.61 ± 0.67*
Wing length FA	0.380 ± 0.07	0.560 ± 0.09
1989		
Thorax length	0.9481 ± 0.01	0.9653 ± 0.01*
Wing length	46.75 ± 0.60	46.16 ± 0.57
Wing length FA	0.689 ± 0.04	0.93 ± 0.04*
Aristal branches	7.3100 ± 0.18	7.300 ± 0.17
Aristal branch FA	0.9000 ± 0.12	1.2000 ± 0.12*
Head bristles	9.8617 ± 0.09	9.6800 ± 0.08
Head bristle FA	0.3617 ± 0.09	0.4800 ± 0.08
Sternopleural bristle	12.9100 ± 0.23	13.20 ± 0.21
Sternopleural bristle FA	1.1000 ± 0.15	1.20 ± 0.15

**P* < 0.05.

simulans. Males were seen to stand in isolation and scissor both wings creating a highly reflectant flashing. They were seen to perform the 'flashing' behaviour for up to 15 min despite the absence of other conspecifics nearby. Males also exhibited aggression to other males on the same piece of fruit. Although there were far fewer *D. pseudoobscura* at the site than *D. simulans*, a higher proportion were found copulating.

As in *D. simulans*, wing and thorax lengths were highly correlated ($r=0.6965$, $P<0.0001$). However, no difference was observed between copulating and single males for thorax length, wing length or arisal branch number in either year (Table 3). Wing fluctuating asymmetry was significantly greater in the single male category in both years. No difference in arisal branch FA was detected in the 1 year it was measured. Thus, in *D. pseudoobscura* males, greater developmental stability rather than size was associated with mating success.

Drosophila mojavensis

As reported earlier by Markow & Toolson (1990), matings tended to occur on cactus arms away from necrotic ooze. Females were observed to fly to these sites after males had settled at them and the movement of the arriving females triggered males to court them. Many matings were observed, although the spiny setting was not always conducive to aspirating flies. The only difference between mating and single males in this species was in thorax length, which was greater in mating males (Table 4). Curiously, thorax length and wing length were uncorrelated ($r=0.3201$, $P=0.184$).

Table 3 Characteristics of single and mating males in a natural populations of *D. pseudoobscura*

	Single male	Copulating male
1988		
Thorax length	1.0377 ± 0.0108	1.0356 ± 0.0124
Wing length	67.45 ± 0.667	68.35 ± 0.772
Wing length FA	0.660 ± 0.097	0.050 ± 0.086*
1989		
Thorax length	1.0855 ± 0.0091	1.0834 ± 0.0115
Wing length	59.855 ± 0.4811	60.011 ± 0.7762
Wing FA	0.1111 ± 0.0473	0.0217 ± 0.0217*
Aristal branches	7.1111 ± 0.0708	7.2282 ± 0.7725
Aristal branch FA	0.4440 ± 0.0813	0.3261 ± 0.6988

**P* < 0.05.

Mating and single males did not differ in fluctuating asymmetry.

Discussion

Mating males were larger than single males in two of the three species. Each species showed a different pattern of association between mating status and morphological phenotype. Mating males were larger in *D. simulans* and showed reduced developmental stability, in keeping with the first prediction. Single and mating *D. pseudoobscura* males were the same size but mating males were characterized by greater developmental stability, which was consistent with the second prediction. The observations on *D. mojavensis*, how-

Table 4 Characteristics of single and mating males from a natural population of *D. mojavensis*

	Single males (22)	Mating males (22)
Thorax length	0.879 ± 0.009	0.923 ± 0.008*
Wing length	27.28 ± 0.344	28.20 ± 0.402
Wing FA	0.905 ± 0.209	0.895 ± 0.263
SP bristles	26.545 ± 0.491	26.364 ± 0.743
Bristle FA	1.273 ± 0.239	1.545 ± 0.235

* $P < 0.05$.

ever, were unexpected. Mating males were larger than single males but no other differences were apparent. The observed absence of any correlations for fluctuating asymmetry in different characters was expected, based upon earlier work (Van Valen, 1962).

Each of the three species examined here differ dramatically in their mating systems and ecology and these differences must ultimately influence the nature and intensity of sexual selection. The distribution of male and female *D. simulans* on fallen fruit is very similar to that reported by Markow (1988) for its sibling species *D. melanogaster*. There was no size difference for *D. melanogaster* between mating and single males found on decaying fruit. Males collected from the skin of unbroken pieces of fruit were significantly smaller, however, suggesting that male size is important in determining who has access to the sites where receptive females are feeding. Male size in *D. simulans* is clearly an important determinant of which males on the same piece of fruit actually mate. Copulating males are not only larger but as a group show a decrease in developmental stability. This observation is consistent with the existence of directional sexual selection on size or some other quantitative trait. It is likely, however, that the advantage of large size for mating is countered by opposing selection on development time as described by Wilkinson (1987) in *D. melanogaster*. Female oviposition behaviour supports the idea that rapid development is advantageous. We observed females ovipositing immediately on fresh fallen fruit and it is clear from the speed at which the fruit decomposes that rapid oviposition is necessary in order for larval development to finish before the fruit dries up.

Drosophila pseudoobscura males who were found mating were no larger on average than single males but showed superior developmental stability as reflected in wing length FA. There may be certain conditions under which larger males experience an advantage, as Partridge *et al.* (1987b) found in approximately half of their collections on bait buckets that mating males were significantly larger. Baits are likely to provide a different sort of arena for sexual selection than pieces of

fruit. In addition, populations may have different age structures on different days and male age is known to influence mating success (Long *et al.*, 1980). Judging from the excess of males at the mating sites and the 'flashing' behaviour of males, it may be that sexual selection acts more strongly on certain wing attributes than on overall size. For example, wing symmetry may be important in the ability to deliver some signal to other males or to females.

Drosophila mojavensis male size may confer some advantage in male-male competition within the lek where the sex ratio is highly male biased (Markow & Toolson, 1990). However, the larger males show no decrease in developmental stability. Unlike the other species, males in *D. mojavensis* are not sexually mature until they are 8-days-old (Markow, 1982), thus natural selection has a longer opportunity, compared to the other two species, to operate on males. The Sonoran desert is a harsh environment and all males who exhibit developmental instability may not reach the age of reproductive maturity. Large size may also confer an advantage in reducing water loss (Markow & Toolson, 1990), providing females with some indication of relative male quality. Receptive females come to male arenas located away from feeding sites and oviposition sites. As they are coming to mate and not to feed and oviposit, they are different from the population of feeding females encountered by *D. simulans* or *D. pseudoobscura* males. Additional and important differences exist between *D. mojavensis* and the other two species. Daily female remating (Markow, 1982) increases mating opportunities and the significant male ejaculatory investment (Markow & Ankney, 1984) and mate discrimination by males (Markow, 1982) suggest that the nature and intensity of sexual selection are very different in *D. mojavensis*.

Reduced size variability in mating compared to single males has been reported for *Drosophila buzzatii* (Santos *et al.*, 1988; Ruiz *et al.*, 1991), *D. sylvestris* (Boake, 1989) and *D. melanogaster* (Markow & Sawka 1991), which suggests that stabilizing selection has a role in courtship success in these species. The data presented here are also compatible with the action of balancing sexual selection. In all three species examined in the present study, the largest as well as the smallest males were found in the single male groups.

Note that field studies differ from laboratory experiments in several respects. We cannot control in nature for male age or mating experience, both of which are known to influence male success (Markow, 1987). In addition, laboratory experiments are typically conducted with virgin females. We know from earlier studies (Gromko & Markow, 1991; T. Markow, unpublished observations) that nearly 100 per cent of females

in natural populations of these species are already inseminated at any given time and that the majority of observed copulations, which are infrequent considering the large number of flies at the breeding sites, represent rematings by females. It is possible, then, that the differences we detect between mating and single males are important for rematings but that virgin matings, if they could be selectively observed, might reveal a different pattern of factors associated with male success.

Finally, the resource ecology of these three species may vary seasonally or with different hosts, creating different suites of selective forces on characters related to mating success. Just as caution should be used in extrapolating from laboratory experiments to natural populations, observations in nature will be most informative if made in more than one setting.

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