

Mate sampling and the sexual conflict over mating in seaweed flies

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The order in which females encounter, or sample, males in a population may have important consequences for mate choice, with the information gathered about males influencing both the preference function and degree of choosiness of females. Sexual selection may be affected as a result. Sampling of particular subsets of males may be a crucial component of individual variation in mate preferences within populations. However, the sequence in which males are sampled may also be important in species without traditional, active mate choice, such as when sexual selection involves sexual conflict over mating. This would occur if the likelihood of a female mating with a male of a certain phenotype changes as a result of previous encounters. We examined the effects of encountering males differing in body size, a sexually selected phenotype, in the seaweed fly *Coelopa frigida*. Sexual selection occurs in this species as a result of a sexual conflict over mating. We show that the outcome of the sexual conflict is independent of the order in which males are encountered by female seaweed flies, with the overall mating advantage to large males being unaffected. In addition, we explored female preference functions and evaluate the heterogeneity in female willingness to mate. We suggest that consideration of mate sampling theory is valuable when examining mate choice in species in which sexual selection is driven by sexual conflict. *Key words:* *Coelopa frigida*, mate choice, mate sampling, seaweed flies, sexual conflict, sexual selection. [*Behav Ecol* 13:83–86 (2002)]

Choosing a mate involves gaining information about potential mates and then making a decision based on this information (Wittenberger, 1983). Information gathering involves some form of sampling, with tactics based either on examining a sample of mates and choosing from that sample (pooled comparisons, including “best of n ”) or on a specific threshold of mate quality by which each individual is evaluated (such as sequential search; reviewed by Reid and Stamps, 1997; Real, 1990; Gibson and Langen, 1996; Wittenberger, 1983). Decision making involves exercising individual mate preferences, typically with females as the choosy sex, limiting the range of males a female mates with.

The information-gathering and decision-making processes are closely associated, together influencing observed patterns of mate choice. For instance, there are two components to mate preferences: the preference function (the relationship between female response and size of stimulus), and the degree of choosiness (effort invested in mate assessment) (Jennions and Petrie, 1997; Widemo and Saether, 1999). Preference functions can be expressed as fixed thresholds for particular male traits. Alternatively, preference functions can be flexible, varying with the males actually available within a population, and thus dependent on the particular phenotypic distribution in the population. If the latter is the case, the way in which females encounter, or sample, males in a population can affect the preference function of those females. Females could adaptively alter mate preference functions in response to their prior experience of males of differing quality and so balance the quality and quantity of mates with strategic mate sampling decisions (Gibson and Langen, 1996; Jennions and Petrie, 1997; Wagner, 1998). In addition, the amount of time

and energy a female invests in choosing mates (e.g., the time assigned to search for and assess potential mates) can interact with the preference function and vary with the particular males encountered. Certain males could limit further searching due to costly behavioral interactions. Mate preferences would again differ among females in response to the specific subset of males a female encountered. Empirical evidence for “previous male effects” of these kinds comes from several species, including mottled sculpins (Downhower and Lank, 1994), sticklebacks (Bakker and Milinski, 1991), and zebra finches (Collins, 1995).

Phenotypic plasticity in mating preferences has obvious consequences for the strength and direction of sexual selection within a population and may be an important component of within- and between-population variation in preferences (Jennions and Petrie, 1997). Crucially, these considerations apply equally to mating systems characterized by active female mate sampling (e.g., mating systems where females visit displaying males in turn, such as lekking species; Höglund and Alatalo, 1995), as well as systems in which females encounter males serendipitously without active searching or sampling tactics. Both situations result in mate-choice decisions being based on previously encountered males as well as the current male. Even if females only sample males passively, choosiness and acceptance criteria could depend on male phenotypes encountered.

Preference functions do not necessarily depend on active mate choice because sexual conflicts over mating can also lead to female behaviors that bias male mating success (Clutton-Brock and Parker, 1995; Parker, 1979, 1983). The importance of sexual conflict over mating leading to sexual selection is now well appreciated, particularly in insects (Rowe et al., 1994; see also reviews in Choe and Crespi, 1997, especially Brown et al., 1997). For species in which mate choice is a result of sexual conflict, we need to know whether the order in which males of particular phenotypes are encountered influences the outcome of the sexual conflict. Possible effects include flexible preference functions, coupled with the costs accrued through interactions with males (such as the buildup

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Received 25 October 2000; revised 22 March 2001; accepted 22 March 2001.

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of costs of rejecting unsuitable males). If females vary their willingness to mate by altering the intensity or duration of rejection responses, then a full description of the selection pressures on males, and the variation in preferences among females, must include these sampling effects.

In the seaweed fly, *Coelopa frigida*, sexual selection on males occurs as a result of a sexual conflict over mating (Day and Gilburn, 1997; Shuker and Day, 2001). The mating system is characterized by scramble competition, with females encountering males effectively at random within the seaweed matrix (see Day and Gilburn, 1997). Both sexes are highly promiscuous, and female receptivity is unaffected by copulation (Shuker and Day, 2001). Mating interactions in seaweed flies involve males mounting females and attempting to force copulation; females usually attempt to prevent copulation with a suite of rejection responses (Day et al., 1990). Larger males are best able to force copulation, so that female rejection creates a preference for large males (Day and Gilburn, 1997). This preference may have evolved as a side effect of selection on females to reduce the number of potentially costly matings (Day and Gilburn, 1997; Gilburn and Day, 1999; Shuker, 1998).

Much work has been done in *C. frigida* describing variation in patterns of mating in natural populations (Crean, 1997; Day and Gilburn, 1997; Shuker and Day, 2001), although the effects of encountering sequences of males on mating patterns have yet to be examined. Here we describe the results of an experiment in which females encountered males of three size classes, with the aim of specifically examining the effects of sampling on the outcome of the sexual conflict. We addressed two questions. First, is there heterogeneity in females' willingness to mate? Previous studies have shown that females' willingness to mate varies at the population level, with average willingness to mate being associated with a chromosomal inversion polymorphism (the $\alpha\beta$ inversion system; Gilburn and Day, 1994, 1999). However, within-population variation in individual willingness to mate has yet to be quantified. Second, do females alter their willingness to mate as they encounter and sample males of different sizes? In other words, do interactions with different-sized males influence subsequent female responses and thus the outcome of the conflict over mating?

MATERIALS AND METHODS

We collected flies as larvae from South Landing, Flamborough Head, Humberside, UK, in August 1996. Adult virgin males and females were aspirated from population cages twice daily during the eclosion period and immediately sexed under CO₂ anesthesia. Females were isolated from males for 2 days at 26°C, with cellulose wadding soaked in 0.5% mannitol solution as a food source. While still anesthetized, we measured each male for left wing length (the standard measure of body size for *C. frigida*; Butlin, 1983), and assigned him to one of three size classes (large, medium, or small). The classes were constructed to maximize size differences. Individual males were kept in isolation for 2 days at 26°C with freshly minced *Fucus* seaweed. All subsequent procedures were carried out without anesthesia.

After isolation, a female was presented sequentially to a male of each size class in a randomly chosen order. The mating chambers were pervaded with the odor of fresh seaweed, but flies did not have access to the weed. Mate trials were carried out at room temperature (22°–24°C). A female had access to only one male at a time. We scored the outcome of the first mount, defined as the male mounted on the female for >5 s (to exclude mount attempts when males did not fully mount) with each of the three males as either a successful

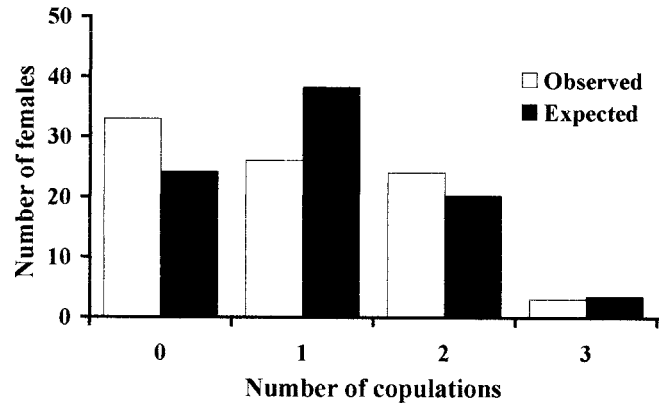


Figure 1

The observed and expected numbers of females involved in 0, 1, 2, or 3 copulations. Expected numbers of females were calculated assuming all females had an equal probability of mating with a male, using the binomial expansion with the observed overall probability of mating: $\text{Pr}(\text{mating}) = 0.346$.

female rejection (ending in the male being displaced from the female), or a copulation (genitalia engaged), termed an "acceptance." We calculated acceptance rates as the proportion of trials in which females copulated. One trial in which male rejection occurred was not included in the analysis. Copulations were allowed to proceed to completion (male genitalia fully disengaged) before the female was removed for presentation to the next male. Females could only copulate once per male, and each male was used with only one female. In total, 86 females were presented to males of all three size classes; an additional 15 females were presented to one or two of the three classes (but these trials have been excluded from analyses involving mating order).

We analyzed the effects of male size class and order of presentation by log-linear analysis, with a multinomial data distribution, using SPSS 7. Because each female was used to produce three data points, we needed to take this non-independence into account. SPSS has two options for the underlying distribution of data for a log-linear analysis. These are either a Poisson distribution or a multinomial distribution. If data cells are not fully independent, then the latter should be used. Other statistics were calculated by either SPSS 7 or Statview 5.

RESULTS

The mean wing lengths of males in the three size classes were very distinct (mean \pm SE; large males: 5.38 ± 0.02 mm; medium males: 4.77 ± 0.02 mm; small males: 4.13 ± 0.02 mm; ANOVA: $F_{2,279} = 840.7$, $p \ll .001$). Mean wing length of females was 4.77 ± 0.04 mm. The overall female acceptance rate was 34.6%, and female acceptance rates did not differ between the first, second, and third mounts (log-linear analysis: $\chi^2_2 = 1.04$, $p = .59$; range 30.2–36.5% acceptance). The probability of a male mating was therefore independent of temporal order. However, females differed in the number of copulations performed, with some females avoiding copulation and others mating with all three males. Using the overall mean acceptance rate as an expected willingness of females to mate, we could examine whether there is heterogeneity between females in the number of males accepted, calculating expected numbers of females with 0, 1, 2, or 3 copulations from a binomial expansion (Figure 1). There was significant heterogeneity between females (chi-square test: $\chi^2_3 = 7.98$, $p < .05$), which was not associated with female body size (ANOVA: $F_{3,83} = 1.09$, $p = .36$). More females than expected did not mate, and fewer females than expected mated only once.



Figure 2
Female acceptance rates (%) with respect to male size class for: (a) all females, including those that did not mate ($n = 101$); (b) only those females that mated twice ($n = 24$); (c) only those females that mated once ($n = 26$). L = large, M = medium, S = small males.

Female acceptance rates differed between the three male size classes (log-linear analysis: $\chi^2_2 = 29.5$, $p \ll .001$; Figure 2a): large males were more often accepted than medium males, who in turn had greater mating success than small males. Within each male size class, there were no differences in size between successful and unsuccessful males (nested ANOVA: $F_{3,275} = 0.9$, $p = .44$), probably a consequence of the range of sizes within each category. Females who mated twice were more likely to mate with large and medium males than with large and small or medium and small males (G test: $G_2 = 43.6$, $p \ll .001$; Figure 2b), whereas females who mated only once were more likely to mate with larger males ($G_2 = 11.3$, $p = .004$; Figure 2c). However, three females mated only with a small male.

If female behavior changes with respect to the male phenotypes previously encountered, we expect a significant interaction between the effects of order and male size class; however, there was no such interaction (log-linear analysis: $\chi^2_4 =$

5.6, $p = .23$). This lack of interaction indicates that male success is unaffected by female experience and that variation between females in the number of copulations performed is not associated with the order in which males of differing sizes are encountered.

DISCUSSION

The processes of information gathering and decision making are central to sexual selection by active mate choice. How information is gathered and then used in mate choice can influence the outcome of sexual selection by female choice (Seger, 1985; Zuk et al., 1990). In addition, individual differences in mate sampling and mate preferences lead to variation in the strength and direction of sexual selection (Andersson, 1994; Jennions and Petrie, 1997; Widemo and Saether, 1999). We suggest that similar considerations are needed in systems without active mate choice, such as when choice is the result of sexual conflict over mating. Conflict-driven sexual selection may also vary if females alter their behavior in response to the particular males they meet.

From the results reported here, it is clear that male mating success in seaweed flies is unaffected by the phenotypes of males previously encountered by females and that variation in overall female willingness to mate is similarly independent of the sequence in which males of different sizes are encountered. The information gathered by females during previous encounters therefore does not influence subsequent interactions. The mating system and the sexual conflict over mating effectively create a situation analogous to sequential search with a fixed acceptance threshold. There has been much discussion of how to study sampling strategies empirically in species with active mate choice (Reid and Stamps, 1997; Valone et al., 1996; Wiegmann et al., 1996); it is important to extend this work to species in which there are sexual conflicts over mating.

Although we used only three size classes, we can begin to consider the decision-making process and the shape of the female preference function in seaweed flies. Generally, the pattern of high to low mating success across the three size classes was consistent among females, with females who mated once or twice generally mating with the larger two size classes. These data are consistent with the preference function being a continuous relationship with male size, which is perhaps what we would expect given the nature of the sexual conflict over mating in this species. However, further work will be necessary to fully describe individual preference functions. For instance, the importance of certain individuals, albeit few of them, mating only with small males needs to be considered. Such data highlight the importance of examining preference functions at both the individual and the population level (Arnold, 1983; Wagner, 1998). There was also individual heterogeneity in females' willingness to mate, with an excess of females who did not mate at all, and fewer females than expected who mated only once. Thus far, female receptivity in this species has only been examined as a population phenomenon, and individual variation has not been quantified. Our data suggest that similar experimental techniques could be used to further examine individual female mating propensity, allowing a more informative genetic analysis of mating behavior.

Coelopa has been well studied in terms of the variation in strength and direction of sexual selection on male size (reviewed by Day and Gilburn, 1997). Much of this work was carried out using single mounts in which virgin males and females were paired once and scored for either acceptance or female rejection (e.g., Gilburn et al., 1992). Variation between populations was evident, but individual variation was not examined. Recent work has revealed that multiple interactions

between pairs of males and females yield consistent mating interactions and repeatable mount outcomes (Shuker and Day, 2001). The results described here extend our understanding of multiple mating interactions by showing that the nonrandom mating produced by the sexual conflict over mating is independent of the order in which females encounter males of differing body size.

The importance of sexual conflicts over mating as a template for sexual selection, especially among insects, is becoming clear (e.g., Brown et al., 1997; Crean and Gilburn, 1998; Rowe et al., 1994). This is the first time that the order in which males are encountered has been examined in a system based on sexual conflict. It is important for such systems to be considered in similar ways to more traditional mate-choice scenarios.

We thank Caroline Crean, André Gilburn, Lisa Hodgkinson, and Ray Wilcockson for their help and assistance. Allen Moore and Richard Preziosi provided many helpful comments on the manuscript, as did Innes Cuthill and two anonymous referees. D.M.S. was supported by a University of Nottingham Studentship.

REFERENCES

- Andersson M, 1994. Sexual selection. Princeton, New Jersey: Princeton University Press.
- Arnold SJ, 1983. Sexual selection: the interface of theory and empiricism. In: Mate choice (Bateson P, ed). Cambridge: Cambridge University Press; 67–107.
- Bakker TCM, Milinski M, 1991. Sequential female choice and the previous male effect in sticklebacks. *Behav Ecol Sociobiol* 29:205–210.
- Brown WD, Crespi BJ, Choe JC, 1997. Sexual conflict and the evolution of mating systems. In: The evolution of mating systems in insects and arachnids (Choe JC, Crespi BJ, eds). Cambridge: Cambridge University Press; 352–377.
- Butlin RK, 1983. The maintenance of an inversion polymorphism in *Coelopa frigida* (PhD dissertation). Nottingham, UK: University of Nottingham.
- Choe JC, Crespi BJ, 1997. The evolution of mating systems in insects and arachnids. Cambridge: Cambridge University Press.
- Clutton-Brock TH, Parker GA, 1995. Sexual coercion in animal societies. *Anim Behav* 49:1345–1365.
- Collins SA, 1995. The effect of recent experience on female choice in zebra finches. *Anim Behav* 49:479–494.
- Crean CS, 1997. Variation in female mate preference in the seaweed fly, *Coelopa frigida* (PhD dissertation). Nottingham, UK: University of Nottingham.
- Crean CS, Gilburn AS, 1998. Sexual selection as a side effect of sexual conflict in the seaweed fly, *Coelopa ursina* (Diptera: Coelopidae). *Anim Behav* 56:1405–1410.
- Day TH, Foster S, Engelhard G, 1990. Mating behavior in seaweed flies (*Coelopa frigida*). *J Insect Behav* 3:105–120.
- Day TH, Gilburn AS, 1997. Sexual selection in seaweed flies. *Adv Study Behav* 26:1–57.
- Downhower JF, Lank DB, 1994. Effects of previous experience on mate choice by female mottled sculpins. *Anim Behav* 47:369–372.
- Gibson RM, Langen TA, 1996. How do animals choose their mates? *Trends Ecol Evol* 11:468–470.
- Gilburn AS, Day TH, 1994. The inheritance of female mating behaviour in the seaweed fly, *Coelopa frigida*. *Genet Res Camb* 64:19–25.
- Gilburn AS, Day TH, 1999. Female mating behaviour, sexual selection and chromosome I inversion karyotype in the seaweed fly, *Coelopa frigida*. *Heredity* 82:276–281.
- Gilburn AS, Foster SP, Day TH, 1992. Female mating preference for large size in *Coelopa frigida*. *Heredity* 69:209–216.
- Höglund J, Alatalo RV, 1995. Leks. Princeton, New Jersey: Princeton University Press.
- Jennions MD, Petrie M, 1997. Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev* 72:283–327.
- Parker GA, 1979. Sexual selection and sexual conflict. In: Sexual selection and reproductive competition (Blum MS, Blum NA, eds). New York: Academic Press; 123–166.
- Parker GA, 1983. Mate quality and mating decisions. In: Mate choice (Bateson P, ed). Cambridge: Cambridge University Press; 141–166.
- Real LA, 1990. Search theory and mate choice. I. Models of single-sex discrimination. *Am Nat* 136:376–404.
- Reid ML, Stamps JA, 1997. Female mate choice tactics in a resource-based mating system: field tests of alternative models. *Am Nat* 150:98–121.
- Rowe L, Arnqvist G, Sih A, Krupa JJ, 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends Ecol Evol* 9:289–293.
- Seger J, 1985. Unifying genetic models for the evolution of female choice. *Evolution* 39:1185–1193.
- Shuker DM, 1998. Sexual selection and sexual conflict in the seaweed fly, *Coelopa frigida* (PhD dissertation). Nottingham, UK: University of Nottingham.
- Shuker DM, Day TH, 2001. The repeatability of a sexual conflict over mating. *Anim Behav* 61:755–762.
- Valone TJ, Nordell SE, Giraldeau L-A, Templeton JJ, 1996. The empirical question of thresholds and mechanisms of mate choice. *Evol Ecol* 10:447–455.
- Wagner WE, 1998. Measuring female mating preferences. *Anim Behav* 55:1029–1042.
- Widemo F, Saether SA, 1999. Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. *Trends Ecol Evol* 14:26–31.
- Wiegmann DD, Real LA, Capone TA, Ellner S, 1996. Some distinguishing features of models of search behavior and mate choice. *Am Nat* 147:188–204.
- Wittenberger JF, 1983. Tactics of mate choice. In: Mate choice (Bateson P, ed). Cambridge: Cambridge University Press; 435–447.
- Zuk M, Johnson K, Thornhill R, Ligon JD, 1990. Mechanisms of female choice in red jungle fowl. *Evolution* 44:477–485.