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Authors: Luck, Nathalie, and Joly, Dominique

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## Sexual selection and mating advantages in the giant sperm species, *Drosophila bifurca*

Nathalie Luck and Dominique Joly

Laboratoire Populations Génétique et Evolution, CNRS – UPR 9034 – Avenue de la Terrasse,  
F - 91 198 Gif sur Yvette Cedex France  
Dominique.Joly@pge.cnrs-gif.fr

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### Abstract

Mate choice may be exercised by either sex; however, females are generally choosier than males because they invest more in their gametes. Female choice is often based on direct benefits, such as better reproductive output, whereas male choice appears to be based on differences in female fecundity. However, when gamete production is limited, sexual selection theories predict that mate choice will be decisive for reproductive success in both sexes. Here, we investigate how mating advantage is achieved in *Drosophila bifurca*, a giant sperm species in which both sexes produce only a few gametes. Our initial expectations were as follows: (1) females would discriminate against sperm-depleted males to avoid fertility cost; and (2) males would discriminate against inseminated females to reduce sperm competition and increase the assurance of paternity of individual gametes. Differences in courtship behaviors were analyzed with regard to the sexual maturity, which is reached after 22 days in males at 21°C, and the reproductive history of both sexes (inseminated versus virgin for females, and sperm-depleted versus sperm-loaded for males). Our results show that: (1) sexual immaturity precludes mating in both sexes; (2) virgin females do not discriminate between sperm-loaded and sperm-depleted males, and (3) males mate preferentially with virgin females, because inseminated females fend off the male, which tended to bring male courtship to an end. Female remating was limited, but increased significantly when the first male was sperm-depleted. Contrary to our initial expectations, these findings suggest that male sperm depletion does not affect female mating preference, whereas the success of male courtship is driven by female behavior. The possibility that female remating was only promoted in response to low sperm transfer is discussed in relation to the gametic system of this species.

**Keywords:** copulation duration, frequency of remating, reproductive behavior, sexual maturity, sperm-limited species

### Introduction

Sexual selection is differential reproduction owing variation in the ability to obtain mates and fertilized oocytes. A few experimental studies have shown that the optimum strategy for males is often to mate with as many females as possible, whereas the optimum strategy for females is likely to select the most attractive male (Gwynne 1984; Pizzari 2003; Wedell and Ritchie 2004). Mate choice by females has been demonstrated in many species (Andersson 1994; Jennions and Petrie 1997), and the most important signals used in mating recognition are based on an assessment of visual, acoustic or chemical characteristics of the male phenotype. However, in some other species complete role reversal has been observed, with choosy males and competitive females (Bonduriansky 2001).

The characteristics that should enhance sexual attractiveness could include those associated with reproductive effectiveness, such as the quantity and quality of the sperm (Trivers 1972; Sheldon and Ellegren 1999). Despite current interest in these processes (Jennions and Petrie 1997; Kokko et al. 2003; Rudolfson et al. 2005) few studies have clearly attributed the success of males to sperm cues

(Wishart 1984; Blount et al. 2001; Wedekind et al. 2001; Evans et al. 2003). At the postcopulatory level, a number of theoretical and empirical studies of sexual selection have mainly focused on patterns of mating arising from sperm competition (Parker 1998; Simmons 2001; Singh et al. 2002), but there has been little attempt to determine male mating success within populations (Tregenza and Wedell 2000; Carson 2002), especially according to the quantity or quality of the sperm. If males have evolved mechanisms that allow limited numbers of sperm to optimize their lifetime reproductive success (Wedell et al. 2002), it can be expected that females would be choosier in sperm-limited species in order to prevent sub-fertile mating. In the present study, we tested this hypothesis in *Drosophila bifurca*, a sperm-limited species (Méry and Joly 2002). Male and female preferences for partners having various quantities of sperm are analyzed. Sexual maturity, reproductive status, and female remating depending both on her sperm reserves and the number of sperm transferred by the males, were investigated.

Previous studies have shown that the reproduction of *D. bifurca* is cyclic, with alternating periods of copulation and egg laying (Méry and Joly 2002). Female egg laying occurs briefly, over a couple of hours, and the number of eggs corresponds to the

number of ovarioles. Once oviposition has been completed, sperm within the female storage organs has been totally exhausted. Consequently, females would then be expected to mate again in order to maximize production of fertile eggs. However, when females mate with twice-mated males, the number of sperm transferred is smaller than the number of oocytes available to be fertilized. As a result, females that have mated with twice-mated males suffer a loss of progeny. To optimize their reproductive output, such females should either increase the remating frequency when sperm transfer is low, or discriminate between males before copulation in terms of the respective sperm supply. These phenomena could occur simultaneously or separately.

## Materials and Methods

### *Flies and rearing*

The *D. bifurca* strain used was obtained from the National Drosophila Species Resource Center, Bowling Green, Ohio (stock n° 1508561621.0). The flies were reared on standard cornmeal-agar-molasses food, at a sex ratio of approximately 1:1. Virgin flies were collected as they emerged, anaesthetized using CO<sub>2</sub>, and sexed. Twenty individuals of each sex were maintained with food in 8 ml vials until used. The mean life span of this species in the laboratory is about 9 months (Méry and Joly 2002). The females take 10 days to reach sexual maturity, and their reproductive potential was determined as described in the following section. In males, sexual maturity was determined after checking the age at which the number of sperm found in the seminal vesicles reaches a plateau, because sexually immature males do copulate when they are singly placed with virgin females in non-competitive trials. Under our laboratory conditions (21 °C), males reached sexual maturity at 22 days in this species (Méry and Joly 2002).

Experiments were done at room temperature, and 25 repetitions at least were performed for each type of cross unless otherwise specified in the figure legends.

### *Effect of sexual maturity on mating*

This first experiment was designed to investigate the effect of sexual maturity on mate preference. Female maturity was investigated after introducing a young (7 day old) and an old (15 day old) virgin female with a virgin (30 day old) male in a mating chamber (standard *Drosophila* 8 ml vials containing corn meal medium). The first copulating partner was registered. Inversely, a young (15 day old) and an old (30 day old) virgin male were kept into vials with a virgin (15 day old) female. Each test lasted 2 hours and was performed in the morning. In order to discriminate between individuals of the same sex, but with differing reproductive status, we marked flies of each type by means of a small hole in the distal part of the wing. This was done 4 days before the experiment, and performed under a binocular microscope with very thin needles after lightly anaesthetizing the flies. This procedure did not bias mate choice for young males ( $\chi^2 = 0.727$ ,  $df = 1$ ,  $P = 0.393$ ), old males ( $\chi^2 = 0.040$ ,  $df = 1$ ,  $P = 0.841$ ), or old females ( $\chi^2 = 0.333$ ,  $df = 1$ ,  $P = 0.563$ ). While the mating pair was copulating, the non-mated sexual partner was removed by aspiration and identified. During the course of the experiment, the vials were checked every minute and the latency time to copulation, defined as the interval

between the onset of copulation and the time the observer placed the last partner in the mating chamber, and duration of copulation were recorded.

### *Effect of reproductive status on mating*

The effect of female insemination on mating success was investigated by placing a virgin female and a previously mated female (mated 24 h earlier with a twice-mated male) with a virgin male. Conversely, a virgin male and a twice-mated male were placed with a virgin female. The twice-mated males had been mated consecutively with two virgin females just before the experimental mating. These males were used for the experimental mating (their third one), and were expected to transfer fewer sperm than the number of eggs females usually lay in a clutch. The flies used were 1 month of age, to allow for the prolonged phase of sexual immaturity of males in this species. The tests were carried out in the morning and lasted 2 hours. The mating chamber was a standard *Drosophila* 8 ml vial containing corn meal medium. For each trial, the latency time before mating and the duration of copulation were recorded. During copulation, the non-mated sexual partner was removed by aspiration and identified on the basis of the marking procedure described in the previous experiment. We also checked that the marking procedure did not bias mate choice with regard to the mated males ( $\chi^2 = 0.040$ ,  $df = 1$ ,  $P = 0.841$ ), or the mated females ( $\chi^2 = 0.310$ ,  $df = 1$ ,  $P = 0.577$ ).

### *Courtship behavior*

In order to detect male or female behavior patterns that could influence the choice of mating partner, we investigated the courtship behavior of *D. bifurca*. Courtship behavior was recorded under artificial light, using a video camera attached to a television and a video tape recorder. Two groups of flies were used for this experiment. First, we recorded the courtship between virgin couples of sexually mature males and females until copulation ( $n = 25$ ). Then, we recorded the courtship between virgin males and inseminated females (mated 24 h previously with a twice-mated male,  $n = 25$ ). All pairs of flies were observed in the courtship cells of Plexiglas dishes (2.5 cm in diameter and 5 mm depth) containing corn meal medium and covered by a cover glass (30 × 40 mm). The flies were manipulated by aspiration. Courtship was observed for 1 hour, or until the flies had mated. The flies were 1 month of age. The video records were then analyzed following the method of Brown (1964, 1965) noting the succession and duration of various types of behavior. Our experimental equipment did not allow us to record courtship songs.

### *Female remating*

Females were first mated with a virgin or a two-time to four-time-mated male. A virgin male was then enclosed with this female every day for 2 hours in the morning on several consecutive days until remating occurred. Only females that did not lay eggs between the two matings were taken into account in this experiment because females had exhausted their sperm after the egg laying (Luck 2001). Mating was always performed in standard, 8 ml vials containing corn meal medium, and was visually confirmed. The interval between first and second matings (in days), the latency time before copulation (in minutes, scored when both sexes were

introduced into the vial) and the duration of copulation were recorded.

### Statistics

Data were tested for normality. As the data were usually not normally distributed (except for those on the feeding behavior), non-parametric analyses were performed.  $\chi^2$  tests were used to compare mating frequencies between the different categories of flies. The statistical probabilities of the remating frequency were obtained using permutation tests (calculated with statXact). The latency time before copulation and the durations of copulation were assessed by Mann-Whitney or Kruskal-Wallis tests depending on the number of categories that were compared (Statistica). Means reported in the text are given  $\pm$  standard errors (SE).

## Results

### Effect of sexual maturity on mating

Virgin males and virgin females mated exclusively with old flies of the opposite sex ( $\chi^2=46.08$ ;  $df=1$ ;  $P<0.001$ ;  $n=50$ ; and,  $\chi^2=48.07$ ;  $df=1$ ;  $P<0.001$ ;  $n=52$ ; respectively, Figure 1). There was only one example of a young female and a young male copulating. We concluded that young females (7 days) and young males (15 days) are less attractive than old ones, and they can be considered to be sexually immature. The latency time before copulation was  $10.90 \pm 2.17$  min for old females, and  $11.57 \pm 2.14$  min for old males, whereas it was 23.93 min for the young female that mated and 2.50 min for the young male that mated. The values for old flies were not statistically different (Mann-Whitney test,  $Z=1.034$ ,  $df=1$ ,  $P=0.301$ ,  $n=100$ ). The duration of copulation was

$4.77 \pm 0.31$  min for old females and  $4.31 \pm 0.24$  min for old males, whereas it was 2.86 min for the young mated female and 8.25 min for the young mated male. The values for old flies are not statistically different (Mann-Whitney test,  $Z=-1.447$ ,  $df=1$ ,  $P=0.147$ ,  $n=100$ ).

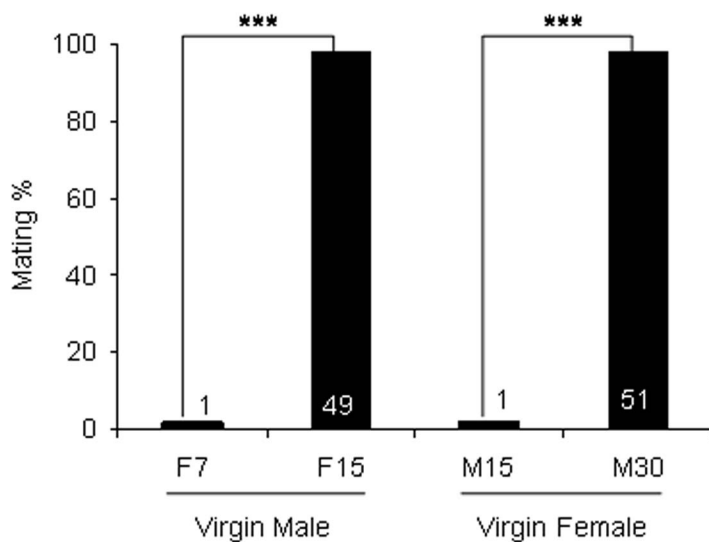
### Effect of reproductive status on mating

Mature virgin males mated preferentially with virgin rather than inseminated females ( $\chi^2=24.00$ ,  $df=1$ ,  $P=10^{-9}$ ,  $n=55$ , Figure 2). However, the values of the mating parameters were not significantly different (the latency time before copulation was  $18.57 \pm 2.97$  min for the virgin females and  $16.95 \pm 6.87$  min for the inseminated females, Mann-Whitney test,  $Z=0.091$ ,  $df=1$ ,  $P=0.927$ ,  $n=55$ , and the copulation duration was  $3.50 \pm 0.29$  min for the virgin females and  $2.95 \pm 0.61$  min for the inseminated females, Mann-Whitney test,  $Z=0.751$ ,  $df=1$ ,  $P=0.452$ ,  $n=55$ ).

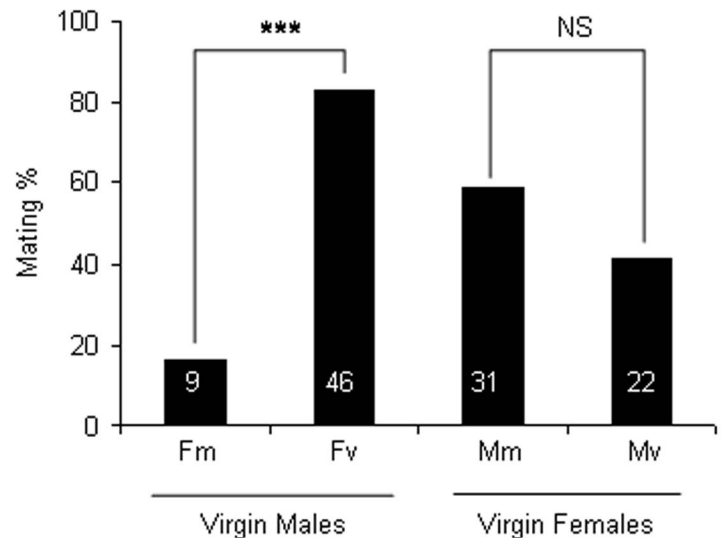
In contrast, mature virgin females did not discriminate between the virgin and mated males ( $\chi^2=1.92$ ,  $df=1$ ,  $P=0.1655$ ,  $n=53$ ; Figure 2). However, the values of the mating parameters were not significantly different in these two groups of males (the latency time before copulation was  $12.86 \pm 3.88$  min for the virgin males, and  $844.22 \pm 3.03$  min for the mated males, Mann-Whitney test,  $Z=0.116$ ,  $df=1$ ,  $P=0.907$ ,  $n=53$ , and the copulation duration was  $5.10 \pm 0.41$  min for the virgin males, and  $4.74 \pm 0.31$  min for the mated males, Mann-Whitney test,  $Z=0.916$ ,  $df=1$ ,  $P=0.359$ ,  $n=53$ ).

### Courtship behavior

The ethogram of the courtship between virgin flies is shown in Figure 3. Courtship is very short and no more than five sequential



**Figure 1.** Effect of sexual maturity on mate preference in *Drosophila bifurca*. The bars indicate the percentages of mating by female (F) and male (M) partners that were young (F7 and M15) or old (F15 and M30) when paired with a sexually mature partner of the opposite sex. The number of flies mated is indicated in the bars. \*\*\* =  $P<0.001$ .

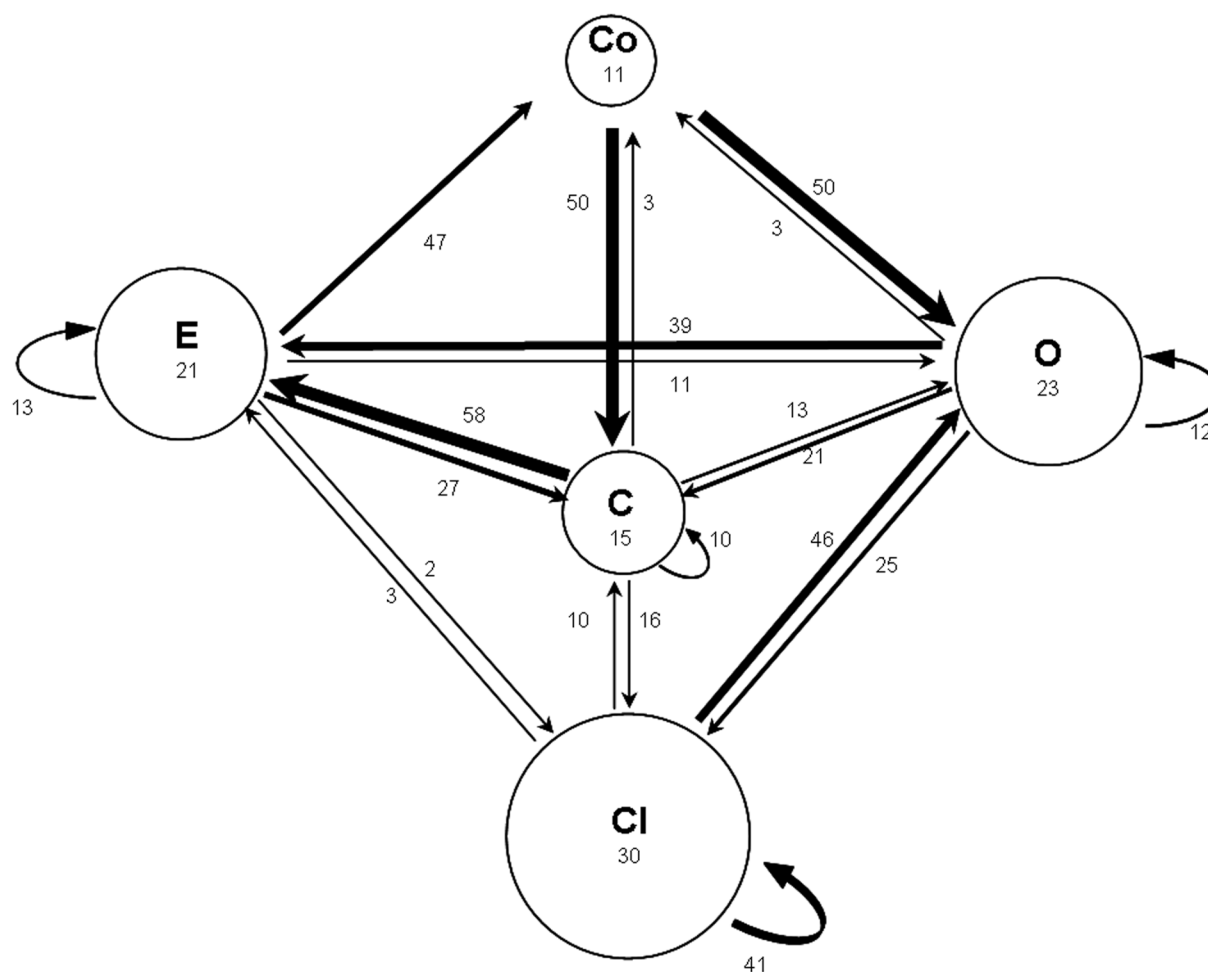


**Figure 2.** Effect of sperm supply on mate preference in *Drosophila bifurca*. The bars indicate the percentages of mating by previously mated (m) or virgin (v) females (F) or males (M). Virgin males mated preferentially with virgin females ( $P<0.001$ ), whereas virgin females mated equally often with mated or virgin males ( $P>0.05$ ). The number of flies mated is indicated in the bars.

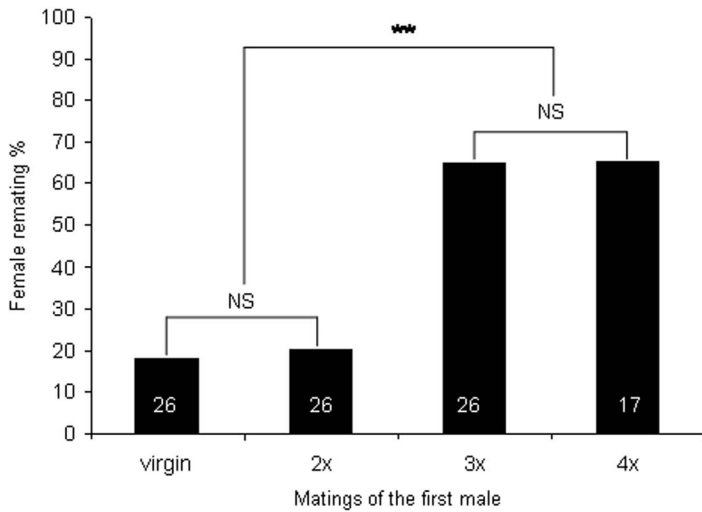
elements of behavior were identified. *D. bifurca* flies are characterized by cyclic mating activity, with intense periods of courtship followed by periods of total immobility. Once the adult flies were placed in the mating chamber, the flies recovered from the aspiration and explored the vials (Cl =  $2.30 \pm 0.50$  min). The males then oriented themselves (O) towards the female's body. At this stage, the male stopped and turned to face the female over a distance of a leg-length. This behavior was followed by circling (C) if the female ran away. Circling involved the male moving around the female, accompanied by extremely brief wing scissoring, i.e. the male opened and closed his wings rapidly. Because scissoring is exhibited exclusively during the circling phase in this species, we have included it in the circling behavior. After circling, or if the female did not run away, the male extended (E) the wing that was nearer the female's head. If the female was willing to copulate, she extended her wings, allowing the male to mount and then to copulate (Co). Courtship lasted on average  $5.50 \pm 0.33$  min ( $n = 25$ ) from

the initial orientation to copulation, with half of the time devoted to grooming. Copulation lasted  $4.40 \pm 0.25$  min ( $n = 25$ ). The small size of the experimental apparatus limited the movements of the flies, but did not seem to affect their behavior, since the mating parameters were very similar to those obtained in previous experiments. Unlike the species of the *D. melanogaster* subgroup, *D. bifurca* males do not approach females from behind or extend their proboscis to touch the females' genitalia. The time spent in wing extensions was extremely short, but appeared to determine whether the females would allow the males to mount or not (Figure 3). If mounting for copulation failed, the males returned to the orientation or circling phase.

Fending behavior by the female systematically terminated male courtship. Female fending consisted of extending one or two legs, generally the middle one, on the side of the body nearest to the male that is being pushed away. This behavior was not included in the male ethogram because it was a solely female response. Female



**Figure 3.** Ethogram of the male courtship between sexually mature virgin *Drosophila bifurca*. The letters indicate the five behaviors observed, and the numbers below each letter correspond to the relative percentages of the behaviors observed during the courtship. The size of the circles is proportionate to the value of the percentages. The arrows indicate the transition between the different behaviors, and their respective widths are proportionate. The numbers along the arrows indicate the relative percentages of the transitions between the different behaviors. Cl = Cleaning; O = orientation; C = circling; E = wing extension; Co = copulation.



**Figure 4.** Percentage of *Drosophila bifurca* females remating depending on the number of times the first male had previously mated. Virgin = males which had not previously mated; 2x-, 3x-, 4x- = the first male had previously mated two, three, or four times. The number of flies mated is indicated in the bars. \*\* =  $P < 0.05$ ; NS = Not statistically significant.

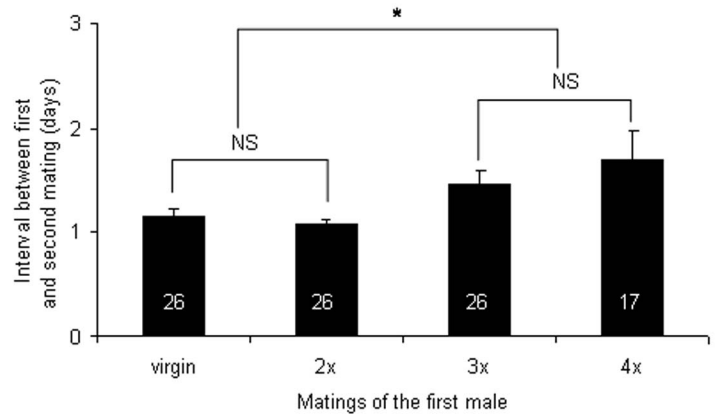
fending is commonly used in other *Drosophila* species to preserve living space between individual flies (Cobb et al. 1985). However, under our laboratory conditions, fending was exhibited by *D. bifurca* in the context of reproduction and was so powerful that males abandoned courtship until the end of the experiment. In most cases, fending occurred after the orientation phase. Females that did not fend off the males were always successfully courted.

In order to disentangle the roles of males and females in successful courtships, we investigated the receptivity of inseminated females to remating with virgin males. Inseminated females fended off males significantly more often than the virgin females did (84% of behaviors instead of 9%,  $t$  test,  $t = -6.726$  and  $P < 0.001$ ,  $n_1 = 25$  and  $n_2 = 25$ ). After being fended off by a female a few times, the males stopped courting. When this occurred, both sexes continued to move around the vial without interacting. Only one of the 25 inseminated females did not exhibit fending behavior, giving rise to a courtship and copulation pattern that was very similar to that observed between virgin partners.

#### Female remating

Remating occurred more often in females that had first been mated with three- or four-time-mated males than in females first mated with a virgin or a twice-mated male (permutation test,  $P < 0.01$ , Figure 4). However, there was no significant difference between females mated with virgin or twice-mated males (permutation test,  $P = 0.6368$ , Figure 4) or between females mated with three- or four time-mated males (permutation test  $P = 1$ , Figure 4).

The interval between first and second mating (Figure 5) revealed no significant difference in the latency time before copulation between virgin and twice mated males (Mann-Whitney test,  $Z =$



**Figure 5.** Interval between first and second mating (mean  $\pm$  SE) of *Drosophila bifurca* depending on the number of times the first male had previously mated. Virgin = males which had not previously mated; 2x-, 3x-, 4x- = the first male had previously mated two, three, or four times. The number of flies mated is indicated in the bars. \* =  $P < 0.05$ , NS = Not statistically significant.

0.476,  $df = 1$ ,  $P = 0.634$ ,  $n = 52$ ) or between three- and four-time-mated males (Mann-Whitney test,  $Z = -0.472$ ,  $df = 1$ ,  $P = 0.637$ ,  $n = 43$ ). However latency times between these two groups were significantly different (Mann-Whitney test,  $Z = -2.280$ ,  $df = 1$ ,  $P = 0.025$ ,  $n = 95$ ). Latency time before copulation and the duration of copulation were not statistically different in the four groups of males (Kruskal-Wallis test,  $H = 4.170$ ,  $df = 3$ ,  $P = 0.243$ , and,  $H = 1.431$ ,  $df = 3$ ,  $P = 0.698$ ,  $n = 95$  respectively, Table 1).

#### Discussion

Females of the sperm-limited species, *D. bifurca*, mate indiscriminately with males irrespective of their mating history. This contrasts to what has been found in *D. melanogaster* (Markow et al. 1978). The mating parameters were similar irrespective of the male and female mating status, and the ability of first-mated males to copulate was not compromised. Although the progeny production potentials of these males are in fact different, the females did not perceive any stimulus that could enable them to select a sexual partner according to his sperm load. However, the females did remate more frequently when the first male had been mated previously. Unlike other species (Singh and Singh 2004), the duration of copulation seemed to be extremely stable, regardless of the reproductive status of the male and female partners involved. In *Drosophila*, the female remating frequency depends on previous sexual experience (Schwartz 1991; Koref-Santibáñez 2001) and the amount of sperm stored which decreases fairly rapidly with the number of eggs laid (Manning 1962; Gromko et al. 1984; Gromko and Markow 1993). However in the present study we confirmed the absence of egg laying between the two matings, and this excluded sperm use as a factor increasing female receptivity to remating. The number of oocytes the *D. bifurca* females could fertilize in one clutch should therefore only have depended on the number of sperm

**Table 1.** Latency time before copulation and duration of copulation (in minutes) for female *Drosophila bifurca* remating depending on the number of times the first male had previously mated. virgin = males which have not previously mated; 2x-, 3x-, 4x- = the first male has previously mated two, three, or four times. N = number of flies tested.

	Latency time before copulation	Duration of copulation	N
virgin	32.54 ± 5.12	6.26 ± 1.30	26
2x	37.02 ± 7.43	5.15 ± 0.43	26
3x	22.01 ± 4.38	5.36 ± 0.40	26
4x	38.53 ± 7.35	4.67 ± 0.41	17

received at copulation. It was previously shown that the number of sperm transferred by the males decreases significantly with the number of previous matings in *D. bifurca* (Méry and Joly 2002), and apparently does so more rapidly than in other drosophilid species (Bundgaard and Barker 2000, and references therein). While these previously mated males can therefore be considered to have been sperm depleted, their future mating success was not impaired.

Female remating is limited in this species, but increases significantly when the first male has been sperm-depleted. A significant difference was found between the second and the third mating groups (Figure 4). However, we would have expected to find a positive relationship with the number of previous mates a male had had. This observation suggests the existence of a threshold above which female receptivity to remating increases dramatically. This leads us to suspect that the ratio between the number of sperm available and the number of oocytes present at the reproductive cycle would influence the female receptivity. The match between sperm and oocyte numbers is crucial for maintaining high progeny production, and anything that would allow females to perceive it would confer a selective advantage. In this case, female receptivity would significantly increase in the context of a low gamete ratio. The detailed analysis of female remating and the number of resident sperm requires further investigation.

Interestingly, the latency time before the second mating increased significantly between the second and the third mating groups, although we would have expected the opposite trend with regard to the percentage of female remating. The results with three- and four-time-mated males are understandable if these males were sperm-depleted and ejaculated less sperm than the female had oocytes to fertilize. Females mated with these types of males needed to remate to complement their sperm stock. However, the data for virgin and twice-mated males are more unexpected, because females mated with these two groups of males could normally be supposed to have received sufficient sperm to fertilize the entire clutch. In the light of the data for female receptivity, and the percentage of rematings after a standard copulation, we surmise that the females which were first mated with virgin or twice-mated males had totally lost the sperm received, and that could explain why they remated sooner than the previous group. The possibility of sperm loss was

checked after dissecting females just at the beginning of remating, prior to the transfer of sperm from the second male. The storage organs of up to 60% of these females (which had first been mated with virgin males) contained no sperm. In this respect, the rapid remating pattern of *D. bifurca* females seems to be very similar to that reported in *D. melanogaster* when females remate within 6 hours (Scott and Richmond 1990; Scott and Williams 1993) despite the great difference of the sperm sizes between these species. Also recent findings from *D. melanogaster* (Snook and Hosken 2004) show that females can release stored sperm from the reproductive tract after copulation with a second male. This process does not depend on the receipt of either sperm or accessory glands proteins from the second male. Then it is assumed that the female mediated processes to discard resident sperm seem to be more frequent than previously thought and appear to concern a wide range of species. The exact role of the second male on the sperm rejection from female tracts in *D. bifurca* needs to be carefully determined in order to highlight the influence of the mating history on female remating.

The one day interval before female remating could be interpreted as a short-term effect of the male accessory gland proteins as it was displayed by the *D. melanogaster* males (Kalb et al. 1993; Wolfner 1997). However, the presence of sperm is indispensable for this refractory period to be extended beyond the first day (Chapman et al. 2003; Liu and Kubli 2003). The contrasting patterns of behavior in virgin and inseminated females from the above experiments, and the very low percentage of inseminated females that remate suggest that a similar physiological mechanism can operate in *D. bifurca*. We can then hypothesize that sperm from the first virgin or twice-mated male partners was lost, preventing accessory gland proteins from inducing the long-term effect of the refractory period, and therefore allowing the females to remate. In the case of a female mated with a three- and four time-mated male, a number of spermatozoa were probably stored in the ventral receptacle, thus allowing accessory gland proteins to induce a longer refractory period.

Courtship behavior in *D. bifurca* is short and involves fewer components than that of other *Drosophila* species (Spieth 1952; Cobb et al. 1989; O'Dell 2003 and references therein). The female reproductive status seems to determine male mating success in this species, since fending behavior by the female ended the male courtship bout. Mated females performed fending at higher rates than virgin ones, so that males copulated preferentially with the latter. Fending is known to occur in a non-sexual as well as a sexual context in *D. melanogaster* subgroup species, and is expressed in response to the proximity of a male (Cobb et al. 1985). It is usually associated with extrusion, abdomen raising, abdomen extension, and abdomen bob behavior to prevent copulation. However, none of these rejecting behavior patterns were observed under our experimental conditions. The use of fending seems to be the major inhibitor of courtship in *D. bifurca*. The effectiveness of rejection behavior suggests that the females influence the timing of remating (Gromko and Markow 1993), and indeed it significantly decreases sexual harassment by the males. Further investigation is required to find out whether this behavior is displayed in the wild.

The advantage of sexually mature flies over immature ones is not unusual in insect species, since a correlation between male mating success and fertility has been identified in a number of species

(Prakash 1967; Markow et al. 1978; Kosuda 1985, but see Brockett et al. 1996). The fecundity of males is known to be related to the number of sperm available and also to a closely correlated parameter, the amount of accessory gland secretions in storage (Spieth and Ringo 1983). We know that the accessory glands of *D. bifurca* are about 10 times larger in mature than immature males (Méry and Joly 2002). Not only the quantity, but also the quality of the seminal substances can vary with age, and the composition of seminal substances is significantly different in mature and immature males in this species (Rohmer C. and Joly D., unpublished data). The composition of cuticular hydrocarbons, which remains to be investigated, could also be involved in the recognition of mature flies, as has already been shown in *D. melanogaster* (Péchiné et al. 1988). In no-choice conditions, immature *D. bifurca* males are able to copulate, whereas they cannot copulate when they are in competition with older ones. Then, the resulting copulation may be due to male-male competition rather than female choice. This suggests that copulation is not exclusively determined by mate choice but is mainly driven by the combination of male and female driven mechanisms. A number of hypotheses have already been proposed to explain such mating traits (Méry and Joly 2002), and the reproductive success of these young males needs to be compared to that young mated males of the same age in order to estimate the potential direct benefit, as has been shown in the Caribbean fruit fly (Teal et al. 2000).

In conclusion, the mating behavior of *D. bifurca* has been shown to be subject to female-driven mechanisms that are potential causes of sexual selection. The mating characteristics of fresh wild flies (kindly obtained from W.J. Etges, unpublished data) show similar patterns to those obtained with the old laboratory strain used here, which suggests that it is a natural phenomenon in this species. Male rejection during courtship is the predominant response of inseminated females. Female remating is limited and likely elicited either by sperm loss or sperm replenishment. It could be that the females, like damselflies (Andrés and Cordero Riva 2000), can estimate the amount of sperm that has been transferred, and handle it according to its quantity or quality. The active role of females in biasing paternity is illustrated by the fact that all copulations in *D. bifurca* lead to sperm transfer, whereas most of the remating females no longer contained any sperm from the first male. We are currently investigating how long after copulation the sperm is exhausted (prior to or after storage). Post-copulatory processes of sperm selection in *D. bifurca* may therefore have a greater impact than pre-copulatory mate choice, even though male sperm competition is not significantly promoted in this species.

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