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Incidence and adaptive significance of multiple mating in females of two boreal *Drosophila virilis* -group species

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The incidence, timing and adaptive significance of multiple mating in female *Drosophila littoralis* and *D. montana* were studied under laboratory conditions. In both species almost all the females were willing to remate within six days, although *D. littoralis* females tend to remate later than the *D. montana* females. Previous copulation or the existence of live sperm in the female storage organs did not reduce female receptivity in *D. montana*. Multiple mating prevented the depletion of sperm stores in both species. The most adaptive reproductive strategy in both species was to defer remating for several days after the initial mating. Even so considerable numbers of females of both species remated within the same day, thus implying some other benefit associated with remating. Experiments with radioactively labelled sperm did not produce any evidence that sperm consumption provides nutrients for female nourishment or egg production. A female of *D. littoralis* could not assess the quality or level of fertility of prospective mates. More diverse offspring did not increase offspring production in either species. Differences in sperm precedence values between different male strains were found in *D. littoralis*. These differences were merely due to competition among sperm from the mating males for access to fertilization rather than preferential sperm utilization by the females. Other probable benefits and costs associated with multiple mating are discussed.

1. Introduction

It has been observed under laboratory conditions that sexual activity and mating are costly to female fruitflies (*Drosophila*) in terms of future survival (Ikeda 1974, Turner & Anderson 1983, Bellen & Kiger 1987, Partridge et al. 1986, 1987, Fowler & Partridge 1989, Partridge & Fowler 1990). The exact mechanism by which sexual activity reduces female survival has remained unidentified, but a number of possibilities have been suggested, including:

1) mechanical damage or adverse physiological consequences due to mating itself or substances transferred during it (Ikeda 1974, Partridge et al. 1986, 1987), and
2) parasite or disease transmission during copulation (Partridge et al. 1987, Fowler & Partridge 1989).

There may also be other potential costs of sexual behaviour in the wild, involving

3) time and energy costs associated with this behaviour (Spieth & Ringo 1983), and
4) increased vulnerability to predators while mating (Manning 1967).

Despite these potential costs of sexual activity, both laboratory and field studies on *Drosophila* reveal that remating is common in this genus (e.g. Gromko et al. 1984a, Harshman et al. 1988, Aspi & Lankinen 1992). The amount of concurrent multiple paternity within the progeny broods in natural populations varies between species and even between populations within one species, but many of the females caught in the wild carry sperm of more than one male at capture. It thus appears that some selective advantages must be attributable to remating to compensate for the costs of sexual activity.

Several hypotheses have been proposed for the adaptive significance of remating (Walker 1980, Thornhill & Alcock 1983, several authors in Smith 1984, Halliday & Arnold 1987), the most obvious explanation being that one mating is not enough for lifetime fecundity and that remating renews an exhausted sperm supply. An increase in productivity resulting from remating has been reported in some *Drosophila* experiments but not in all (see Gromko et al. 1984a, Ridley 1988 for reviews).

Another adaptive explanation for multiple mating is that it provides the females with extra resources for progeny production. It has recently been shown that the males of some *Drosophila* species provide nuptial gifts for the female during courtship (Steele 1986) or directly pass nutrients into the female in the form of the male ejaculate (Markow & Ankney 1984).

Multiple mating may also be a hedge against reduced male fertility, since it allows an inadequately inseminated female to acquire an adequate sperm supply. Infertile first matings are not uncommon in *D. melanogaster* (eg. Mayer & Mayer 1961); in addition to which even one copulation can significantly reduce male fertility even for several hours (Lefevre & Jonsson 1962, Markow et al. 1978). Females of *D. melanogaster* may even be able to recognize the fertility state of the males, for Markow et al. (1978) have shown that they favour fertile males in competition with genetically similar males whose fertility has been reduced due to a previous copulation.

If the female remates before her initial sperm supply is totally exhausted it may lead to differential use of the sperm from successive ejaculates. This phenomenon, called sperm precedence, has usually been interpreted in terms of intrasexual selection among males (Smith 1984, Lewis & Austad 1990), but in fact it may also be due to the female's sperm utilization strategies (Walker 1980, Halliday 1983, Lewis & Austad 1990). The sperm of the first mate can be replenished with sperm from a second one during remating in *Drosophila* (Scott & Richmond 1990), just as there is evidence that the females in other species may use sperm competition as a means to securing a genetically superior mate (Madsen et al. 1992, see also Parker 1992). Thus multiple mating may act as a mechanism of mate choice and enable the female to leave progeny from 'good quality' males (Walker 1980, Halliday 1983, Simmons 1986).

This paper examines the incidence and adaptive significance of multiple mating in females of *D. montana* Stone, Griffen & Patterson and *D. littoralis* Meigen under laboratory conditions. These species are phylogenetically closely related and ecologically rather similar (Lumme et al. 1979). They both belong to the *virilis* species group and are sympatric in northern Scandinavia. Both species are univoltine or almost univoltine in boreal populations. They overwinter as adults, and the mating season in early spring is very short (Lumme et al. 1974, 1978, Aspi et al. 1992). The incidence of multiple insemination among progeny broods of *D. montana* has been studied in the field and appears to be common. Practically all the females caught in the field carried sperm from more than one male (Aspi & Lankinen 1992). The aim of this study is

1) to describe the female receptivity pattern and survey the mechanisms regulating receptivity, and also to investigate
2) whether multiple mating prevents the exhaustion of female sperm stores,
3) whether the male contributes to offspring production in the form of male ejaculate,
4) whether repeated matings reduce male reproductive ability and attractiveness, and finally
5) whether remating is used as a mechanism of female mate choice.

2. Material and methods

2.1. Flies used

The wild type strains were derived from multifemale collections made at a locality near Kemi, northern Finland (65°40′N, 23°35′E; Grid 27°E number 729:39) in May 1990. The cultures were maintained in a population cage at 19°C with continuous light for about four to six months before the experiments.

Two laboratory strains carrying visible autosomal markers were used in the sperm utilization experiments in order to distinguish between the progeny of first and second matings. The *D. montana* strain carrying recessive brownie (Ou *bw*) was obtained from an isofemale line collected from a nearby population (Oulanka; 66°22′N, 29°21′E; Grid 27°E number 736:60). The eye colour of these flies is brownish and the male testes are white as compared with the normal orange. The *D. littoralis* mutant strain with mutant scarlet eyes (359 *st*) originated from Krasnodar (Jekaterinodar), Russia.

The effect of live sperm in the female storage organs on remating was studied in *D. montana*. For this purpose rudimentary testis (*rt*) males, which do not transfer sperm during copulation, were collected from an isofemale strain from Oulanka. These have minute testes, but the accessory glands and seminal vesicles are apparently normal.

The emerging virgin males and females were separated under CO_2 anaesthesia and kept in separate vials until sexually receptive at the age of two (*D. littoralis*) or three weeks (*D. montana*) (see Watabe & Higuchi 1984).

2.2. Incidence and timing of remating

In the remating assay the females were not kept with the males continuously but were given periodic opportunities to remate (see Fuerst et al. 1973, Newport & Gromko 1984). This design more closely represents the mating behaviour of *Drosophila* in the field (see Spieth & Ringo 1983) than continuous access of males.

All the mating experiments were performed in a round petriglass chamber (diameter 5 cm, height 7 mm) with a nylon net lid. The floor of the chamber was covered with a moistened filter paper. For the initial mating, one virgin female was placed together with two wild type males and observed for 50 minutes or until copulation occurred. Non-mated females were discarded. Remating was attempted four hours, three days and six days after the initial mating. Mated females were aspirated into the chamber with two wild type males, and the number remating within 50 minutes was recorded. An opportunity to remate was given only once.

Since both copulation and sperm may influence remating in *Drosophila* (Maynard Smith 1956, Manning 1962, Gromko et al. 1984b, Scott 1987), the effects of these factors were tested in *D. montana* by comparing the frequency of females mating in the initial experiments and those remating, and by comparing the remating frequency between females that had previously mated with *rt*-males or with fertile control males. Remating is affected by both the copulation and the sperm factors in the first case but only by the copulation factor in the latter. Both mating and remating experiments lasted for three hours or until copulation occurred; the remating experiments were performed half an hour after the initial mating.

2.3. Productivity of once- and twice-mated females

Three sets of sperm utilization experiments were conducted to test the effects of multiple mating on female offspring production. The females in these experiments always bore a visible eye colour marker, and the males either had a homozygous marker or were of the wild-type. In the first set of experiments the females were mated only once, either to a wild-type or to a marker male. In the second set, they were mated twice consecutively to the same type of male, and in the third set of experiments they were mated sequentially to a wild-type and marker male in both orders so as to be able to distinguish the progeny sired by the first and the second male. The opportunity to remate was given after three hours, three days and six days. Females who did not remate were discarded.

All the females were transfered to fresh food vials every 3 days for a total of five transfers, and were discarded six days after the fifth transfer. The emerging progeny were counted separately for each transfer representing a three-day egg-laying period, and a vial was discarded when no flies were obtained within six days. The paternity of the offspring was determined from the broods of females mated sequentially to two types of males. Second male sperm precedence (P_2) was calculated for each female in terms of the proportion of the offspring fathered by the second male (Boorman & Parker 1976), and these values were arcsin-transformed for statistical analysis. The level of sperm precedence was also calculated separately for marker and normal males.

Since the duration of copulation may act as a mechanism for reducing the chances of a subsequent effective insemination of the same female by a different male (Parker 1970, Simmons & Parker 1992), or may otherwise affect male fitness (Krebs 1991), durations were measured from a sample of copulations including initial matings and rematings.

2.4. Nutrient transfer through the ejaculate

Whether the male provides nourishment to the female through the ejaculate was tested by mating radiolabelled males with unlabelled females and assaying the transmission of radioactivity to the developing eggs or various parts of the female's body (Boggs & Gilbert 1979, Markow & Ankney 1984, Markow 1985). Some labelled females were also mated to unlabelled males and the radioactivity of the eggs produced was studied.

Twenty to thirty second-instar larvae were placed in vials containing 0.3 g of culture medium mixed with 2.5 μCi or 10 μCi ^{14}C labelled amino acids. The labelled males that emerged were then mated to unlabelled females, which were dissected either immediately or after three to six days after mating in different replicates. The dissected female parts (head, thorax, abdomen, reproductive tract and ovarian eggs) and oviposited eggs were crushed onto a filter paper and autoradiographed.

2.5. Effects of multiple mating on male fertility and attractiveness

Since differences in sperm precedence value were found between genotypes only in *D. littoralis* (see Section 3.3) the experiments were restricted to this species. To study the effects of repeated matings on male fertility a mature male was placed together with two virgin females in a mating chamber, and the mated females were replaced repeatedly with virgin ones. The number of copulations per male was recorded during a two-hour period. The progeny produced by mated females were examined as in the remating studies.

To study the effects of male 'experience' (previous copulations) on female choice, males marked with small wing clippings were initially paired with virgin females in mating chambers. Immediately after the first mating the female was removed and another male was aspirated into the chamber and the males were allowed to settle down. After one minute, one virgin female was aspirated into the chamber and the behaviour of the flies was observed for 40 minutes or until copulation occurred. The identity of the copulating male was recorded. If it was the virgin male, the experiment was terminated, otherwise it was continued until the 'experienced' male had copulated sequentially four times.

2.6. Adaptive significance of female choice

The mate choice hypothesis for remating requires that the female should be capable of assessing the male quality to increase her offspring production, i.e. that female choice is an adaptive one. Male quality is here used in the broad sense (Halliday 1983), including the male genotype, but also other possible male effects on offspring production (e. g. transmission of beneficial micro-organisms during copulation: Starmer 1988). The possible correlation between female reproductive success and the attractiveness of her mate was studied in *D. littoralis* in the course of the experiments concerning the effects of repeated mating on male fertility, the number of matings achieved in the two-hour period being used as a measure of male attractiveness. The analysis was restricted to the first female mated by each male.

3. Results

3.1. Incidence and timing of remating

There were significant differences in remating frequencies with time after the initial mating in both *D. littoralis* ($G_{(2)} = 67.27$; $P < 0.001$) and *D. montana* ($G_{(2)} = 33.40$; $P < 0.001$), almost 40% of the *D. montana* females remating four hours after the initial mating, whereas only a small proportion of the *D. littoralis* females were willing to do so (Fig. 1). The proportion of females remating was approximately doubled in both species after three days, the increase in the proportion between the first and third day being significant in *D. littoralis* but not in *D. montana*. After six days almost all the females were willing to remate in both species, and no difference between the species was observed. The rematings were apparently not forced copulations, since in all cases the females lifted their wings to acceptance posture before copulation.

Previous copulation had only a slight effect (if any) on remating in *D. montana*. In the initial mating trials with normal fertile males, 57% of the females mated within three hours ($n = 74$), while the proportion remating in trials made half an hour after the initial mating was 41% (Table 1). The difference is not significant with these sample sizes ($G_{(2)} = 0.97$; $P > 0.1$). An absence of live sperm in the female storage organs similarly did not influence the proportion of remating in *D. montana*. The remating frequency was similar among females who had mated with *rt* males and with normal fertile males (Table 1; $G_{(1)} = 0.023$; $P > 0.1$). About forty percent of the females did remate within three hours regardless of the genotype of the first male. Latency time to remating was also similar in both experimental groups (Table 1).

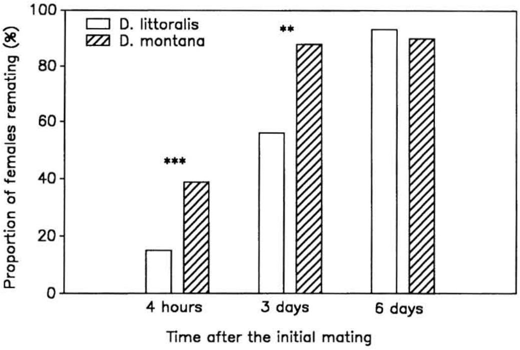

Fig. 1. Proportions of Drosophi*la littoralis* and *D. montana* females remating in trials made four hours ($n = 80$), three days ($n = 50$) and six days ($n = 30$) after the initial mating. Asterisks indicate significant difference between species in chi-square tests: ** = $P < 0.01$, *** = $P < 0.001$.

Table 1. Proportion of *D. montana* females remating with a wild-type male having first mated with a normal fertile male or with an *rt* male, which does not transfer sperm during copulation. The mean latency period (±*SD*) to remating is also presented.

Male genotype	*n*	Females remating (%)	Latency to remating (sec)
+/+	42	41	113.8 ± 53.5
rt/*rt*	23	44	103.7 ± 53.7

3.2. Number of offspring of once and twice-mated females

The male genotypes appeared to be rather similar in fertility in both species (Table 2). There were no differences in the total number of prog-

Table 2. Total number of progeny (±*SD*) produced by once-or twice-mated females mating sequentially with either similar or different types of male in *D. littoralis* ($n = 108$) and in *D.montana* ($n = 75$).

First male genotype	Second male genotype None	Wild	Marker
D. littoralis			
Wild	37.2 ±31.7	63.2 ±34.1	52.1 ±33.0
Marker	41.2 ±24.2	51.2 ±36.1	77.2 ±74.1
D. montana			
Wild	10.5 ± 9.0	20.3 ±17.7	24.5 ±15.3
Marker	22.5 ±16.3	30.1 ±22.8	23.0 ±10.7

eny of once-mated females fathered by the different male genotypes in either *D. littoralis* ($t = -0.4181$; $df = 36$; NS) or *D. montana* ($t = -1.8934$; $df = 15$; NS). In the four groups of twice-mated females the genotype of the first male, second male or any combination of these did not have any effect on the total number of offspring (ANOVA: all F-values nonsignificant). Since no differences were found between the male genotypes in their capacity to leave offspring, the data from the different mating patterns (i.e. one mating per female or two matings per female, in various periods after the initial mating) were combined for the subsequent analysis.

There were significant differences in the mean number of progeny to emerge between transfers (each representing a three-day egg-laying period) both in *D. littoralis* (two-way ANOVA: $F_{4,520} = 64.06$; $P < 0.001$) and in *D. montana* ($F_{4,355} = 44.07$; $P < 0.001$), and also between mating patterns in both species ($F_{3,520} = 3.41$; $P < 0.05$ and $F_{3,375} = 3.36$; $P < 0.05$, respectively). There were also significant interactions between the two factors affecting the mean number of progeny to emerge from a vial in both species ($F_{12,520} = 4.93$; $P < 0.001$ and $F_{12,375} = 3.39$; $P < 0.001$).

The fecundity pattern was rather similar in both species (Fig. 2). If a female mated only once, her number of progeny had already decreased significantly after three days. Fecundity in *D. montana* was nil after six days, whereas the females of *D. littoralis* maintained some fertility until the third transfer (day 9). A second mating four hours after the initial one did not improve the female's reproductive success in either species, but a second mating after three days or six days increased the productivity to the original level. The decline in fertility after the second mating is rather similar to that after the first mating.

The data for *D. littoralis* implied that remating was positively correlated with the rate at which the sperm stores were depleted among the females which mated three or six days after the initial mating. The females which were willing to remate on day three produced significantly more offspring up to this point (mean 26.5; SD = 16.5) than those which remated on day six (mean 15.1; $SD = 14.6$; $t = 2.32$; $df = 50$; $P < 0.05$). In *D. montana*, the difference in progeny production between the females which remated three days (mean 14.4; $SD = 10.9$) or six days (mean 11.8; $SD = 10.4$) after the initial mating was not significant ($t = 0.596$; $df = 30$; $P > 0.10$).

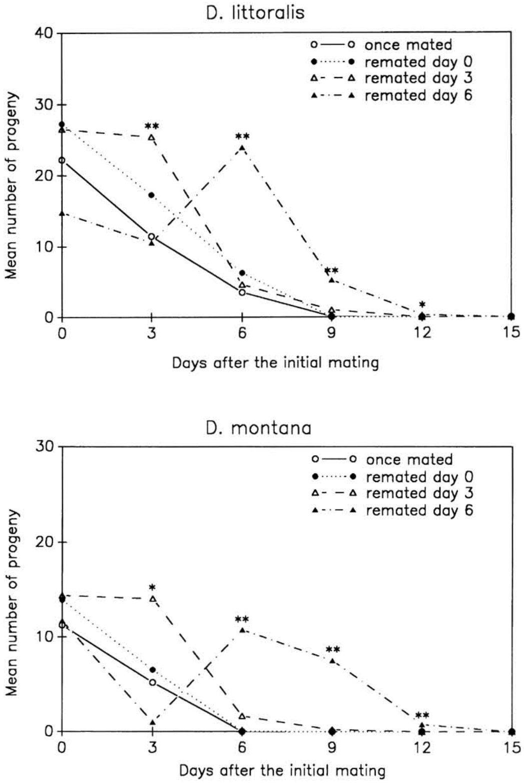

Fig. 2. Mean number of progeny per transfer produced by once-mated or twice-mated *Drosophila littoralis* and *D. montana* females at various intervals after the initial mating. Asterisks indicate significant difference from the mean of the once-mated females in Dunnett's test: * = $P < 0.005$, ** = $P < 0.001$.

Significant differences in the total number of offspring produced during the experiment occurred between the mating patterns (Table 3), although multiple comparison (Dunnett's test) revealed significant differences only between the once-mated females and those which had remated three days after the initial mating.

The sperm precedence pattern was rather similar in both species. If the second mating occurred six days after the initial one, all the progeny following it was sired by the second male. In the remaining cases second male sperm precedence, as measured by mean P_2 averaged over all matings and unweighted by the females's total productivity (Gromko et al. 1984a), was 0.72 (*SD* = 0.35) in *D. littoralis* and 0.68 (*SD* = 0.38) in *D. montana*. A 2 × 2 ANOVA was used to test the effects of the second male genotype and the remating interval on the P_2 values (Table 4). The proportion sired by the second *D. littoralis* male was significantly lower if the remating occurred four hours after the initial mating (P_2 = 0.54; *SD* = 0.44) compared with that after three days (P_2 = 0.81; *SD* = 0.28), whereas in *D. montana* the corresponding shift in the P_2 value between four hours (0.62; *SD* = 0.35) and three days (0.74; *SD* = 0.41) was not significant. Also, the second male genotype affected the sperm precedence value in *D. littoralis*, the overall P_2 value for a wild-type male being 0.63 (*SD* = 0.37), significantly lower than the value for a *st* male, 0.85 (*SD* = 0.30). In *D. montana*, on the other hand, there was little effect of mating order on the proportion of the post-remating progeny sired by each male, the respective P_2 values being 0.67 (*SD* = 0.42) for a wild-type male and 0.70 (*SD* = 0.34) for a *bw* male.

Table 3. Total number of progeny (mean ± *SD*) produced by various twice-mated females compared with once-mated females in *D. littoralis* and *D. montana*. Significance of analysis of variance: * = $P < 0.05$.

Females	*D. littoralis*		*D. montana*	
Once mated	36.0 ±28.4	40	16.9 ±14.0	18
Remated day 0	50.8 ±47.1	15	20.5 ±15.0	25
Remated day 3	57.6 ±38.0	37	30.3 ±21.7	24
Remated day 6	55.1 ±29.1	16	31.8 ±20.4	8
F-value	2.75*		2.77*	

Table 4. Analysis of variance in sperm precedence values with respect to second-male genotype and day of remating in *D. littoralis* (*n* = 38) and *D. montana* (*n* = 34). Significance of analysis: * = $P < 0.05$

		D. littoralis		*D. montana*	
Source	*df*	*SS*	*F*	*SS*	*F*
Day	1	5994.1	5.88*	1083.6	0.83
Genotype	1	4458.8	4.37*	340.2	0.23
Day × genotype	1	1129.3	1.11	2701.8	1.84

The copulations were considerably longer in *D. littoralis* than in *D. montana*, lasting over six minutes (mean 437.1; *SD* = 91.1 sec; *n* = 36) as compared with less than four and a half minutes (261.6; *SD* = 53.5 sec; *n* = 35). There were no differences in copulation duration between the initial matings and rematings in either species. Behavioural observations on *D. littoralis* indicated that there were significant differences in copulation duration between strains (t = 2.114; df = 34; $P < 0.05$), but prolonged copulation could hardly have been the mechanism by which sperm precedence was achieved, as the wild-type males were the ones having the longer copulations (469.4 sec; *SD* = 88.3), not the *st* males (408.1; *SD* = 85.6 sec), which had the more competitive sperm.

3.4. Nutrient transfer through the ejaculate

A significant amount of radioactivity was found in female reproductive tracts immediately after copulation, but the amount of label tracts decreased rapidly, so that while there was still some radioactivity left after five days there was no longer any after six days. Other parts of the body did not show radioactivity at any time, nor were any traces of radioactivity found in the eggs of the unlabelled females mated to labelled males, whereas the label administered to females mated to unlabelled males appeared later in their eggs. The data thus do not give any evidence of the use of the male ejaculate for egg production or female somatic maintenance.

3.5. Effects of rematings on male fertility and attractiveness in *D. littoralis*

D. littoralis males mated repeatedly with new virgin females up to 9 times within a two-hour period. The decline in fertility, as expressed in the mean numbers of offspring produced per mating (Fig. 3), was clear (Spearman rank correlation coefficient $r_s = -1.0$; $P < 0.01$) but slow. The relationship between the number of offspring produced by a female and the number of consecutive matings of her mate appeared to be a

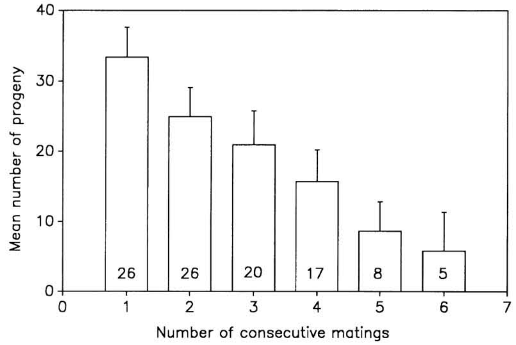

Fig. 3. The mean numbers (+*SE*) of offspring produced per mating by *D. littoralis* males from the first six consecutive matings. The numbers within the boxes indicate the number of progenies studied.

rather linear one, forecasting that the productivity will be nil just after the seventh consecutive mating.

The relative mating success of previously copulated (1–3 consecutive matings) and virgin males in a competitive situation revealed that the females did not copulate selectively with fertile males (Table 5). If any tendency existed it was for the females to show a slight preference for the previously copulated males.

3.6. Adaptive significance of female choice

The choice of mate by the female was not adaptive in this experiment, i.e. their offspring production did not covary with the mating success of their mates. The correlation between the lifetime offspring production of the first female mated by a male and the total number of females mated by the male was not significant ($r = 0.05$; $n = 36$; $P > 0.1$). Even with overall first-mating means, the difference in the number of progeny of males who were capable of attracting only one female (43.5; $SD = 27.9$) vs. multiple-mated males (33.4; $SD = 21.5$) was not significant ($F = 1.03$; $df = 35$; $P > 0.1$).

Table 5. Mating success (%) of virgin and previously copulated (1–3 copulations) *D. littoralis* males in female-choice experiments.

Previous copulations	n	Male chosen copulated	virgin	X^2	*P*
1	54	61	39	2.67	>0.1
2	30	63	37	2.13	>0.1
3	19	47	53	0.05	>0.1

4. Discussion

Females of boreal *virilis* group species showed extensive multiple mating, almost all the females of both species being willing to remate within six days. Significant differences were observed between the species in the timing of this remating, revealing that despite similar environmental circumstances, there could still be differences among closely related species in female decisions concerning reproductive tactics.

Multiple mating prevented the depletion of sperm stores, and the number of progeny produced by females which mated again several days after the initial mating was larger than that produced by the once-mated females. It has also been observed in other *Drosophila* species that one mating is not enough to ensure continuous fecundity (Pruzan-Hotchkiss et al. 1981, Turner & Anderson 1983, Gromko et al. 1984a, Markow 1985). In fact, repeated mating may be universally necessary for full fecundity and fertility in female insects (Ridley 1988).

If the only advantage of remating is renewal of the sperm supply, then the female may optimize sperm utilization best when she remates only after almost all the sperm from the previous copulation has been used (Walker 1980). There is evidence that *D. melanogaster* females tend to remate when all (Manning 1962, 1967) or at least most of the sperm (e.g. Gromko & Pyle 1978, Gromko et al. 1984a) has been exhausted. The females of *D. littoralis* also showed some ability to synchronize their remating to sperm depletion, but no such ability was found in *D. montana*.

The adaptive significance of rapid remating within the same day expressed by females of both species seems to be more questionable. In both species a considerable proportion of the females remated before they had used any of the sperm from the first mating. It is possible that remating within the same day may increase female productivity, although the increase was not significant in the experiments. However, the most adaptive strategy for the female would still have been to defer her remating for several days (see Table 3). The proportion remating within the same day was so large that it is improbable that only those females which were inadequately inseminated in the initial mating were actually remating. Moreover, the absence or existence of live sperm in the storage organs did not affect the remating of *D. montana* females. Other species of the genus *Drosophila* also show rapid remating (e.g. Bundgaard & Christiansen 1972, Fuerst et al. 1973, Markow 1982, 1985) and a lack of ability to synchronize remating to sperm depletion. Most of *D. pseudoobscura* females remate when only approximately 30% of the sperm has been utilized (Pruzan-Hotchkiss et al. 1981), and like the boreal *virilis* group species, *D. hydei* females can also remate sequentially within several hours without any visible gain (Markow 1985). It thus appears that if mating is really costly for the *Drosophila* females, other advantages in addition to increased fertility must be involved in multiple mating.

The mating ecology of the boreal *D. virilis* group species offers theoretical support for some of the potential advantages of remating. A male contribution to oogenesis has been observed in species which live in extreme environments (Markow & Ankney 1984), where male success may critically depend on a nutrient contribution through the ejaculate (Markow 1982, 1985). The environmental circumstances experienced by the boreal *virilis* group species during the mating season indeed are rather extreme, as when the first copulations occur most of the ground is still covered by snow. However, the experiment with radiolabelled sperm nevertheless did not give any evidence of direct nutrient transfer for egg production or for somatic maintenance.

Because the mating season among these species is so short in the field, the risk of some males having reduced fertility due to previous matings is large. Multiple mating experiments with *D. littoralis* males clearly demonstrated that sperm producing capacity or other physiological constraints (see Lefevre & Jonsson 1962) significantly limited the reproductive ability of males, and since the females were not capable of recognizing the state of fertility of the males male sterility may be a potential reason for rapid remating. This is scarcely a plausible explanation in natural populations, however. Given competition with other males and the time and energy costs of finding a receptive female, the maximum number of matings achieved per male in present experiments surely cannot be reached in the wild. When mating frequency is at a lower level than in the present experiments there is no decrease in male fertility at least in *D. littoralis* (Bressac et al. 1991). Thus, in natural circumstances males can probably retain a high fertility despite multiple matings.

Male attractiveness has been shown to affect offspring genetic quality in some *Drosophila* studies (Partridge 1980, Taylor et al. 1987, but see Schaeffer et al. 1984), and also female fecundity in some insects (e.g. McLain & Marsh 1990). The present experiments were planned to examine the male's total effect on offspring production, and do not permit us to distinquish effects on female fecundity and offspring preadult survival. However, no evidence was found that multiple mating is used as a mechanism to increase offpring production. The multiple mating experiment allows evaluation of whether the female controls sperm precedence in favour of good quality males, or whether the differences in sperm precedence between the genotypes are only due to competition between the males for access to fertilization (Walker 1980, Halliday 1983, Lewis & Austad 1990). If the sperm of some genotype were to gain precedence due to female choice and not due to competition between males we would expect the female choice to be revealed in the number of offspring produced. Significant differences in P_2 values between strains were found in *D. littoralis*, where the sperm of *st* males predominated over that of wild-type males. If the precedence of *st* male sperm was due to female choice, this choice did not increase the number of progeny produced in the experiments, since mating order has no effect on total offspring in *D. littoralis*. Neither was the female choice hypothesis supported by the direct test of the female's ability to assess the quality of the males, for the reproductive success of *D. littoralis* females did not covary with the attractiveness of wild-type males.

Given that none of the mechanisms tested here provides a probable explanation for additional mating observed in boreal *virilis* group species, several possibilities are left open for consideration. It may be that

1) remating is not directly beneficial to the females,
2) mating is not costly to the females,
3) multiple mating may involve benefits which were not tested here, and finally
4) mating, even though costly, may still be less costly than rejecting a prospective mate.

Halliday & Arnold (1987) argue that multiple mating may not be beneficial to the female and suggest that, due to the genetic correlation between female and male behaviour, female multiple mating may have evolved as a pleiotropic response to the selection of high mating frequency in males. This new and unprejudiced hypothesis is not supported by comparative data (Sherman & Westneat 1988), or by experimental results (Cheng & Siegel 1990, Gromko 1992, Stamenkovic-Radak et al. 1992) in which no phenotypic or genotypic correlations between male and female mating frequencies have been found. However, as pointed out by Arnold & Halliday (1988, 1992) there may be problems in the interpretation of these experiments, and the hypothesis is still controversial.

In the "sexy-sperm" model (Harvey & May 1989) multiple mating is not directly beneficial to the female either. This hypothesis proposes that multiple-mating females are fertilized by the most competitive sperm, and they have a indirect selective advantage over the single-mating females because their sons are likely to be succesful in sperm competition. Curtsinger (1991) has studied a simple genetic model of this hypothesis, and shown that the conditions for the "sexy-sperm" model to work are very restrictive. Thus sperm competition probably plays no special role in the evolution of female mating frequencies.

We can also ask whether mating is really costly to *Drosophila* females. The costs prior to mating, such as time and energy costs devoted to finding a mate and to courtship, are probably low for *Drosophila* females. Drosophilid mating (except for some Hawaiian and Australian species) takes place "at the restaurant", on decomposing masses of plant material (Spieth 1974, Spieth & Ringo 1983). While feeding, the receptive females have the opportunity of sampling many males with only a small expenditure of energy.

An often cited cost is the increased vulnerability to predation while mating, although there is little empirical evidence to support this (Thorhill & Alcock 1983, Gwynne 1989). Increased vulnerability has been demonstrated in insects only on a few occasions (Wing 1988, Arnqvist 1989, Sih et al. 1990), although many authors have tested the hypothesis experimentally (Gwynne 1989). Although Parsons (1978) and Spieth (see Spieth & Ringo 1983) have suggested that predation may be the major selection pressure which led to the evolution of cryptic lek behaviour among some Hawaiian and Australian species, the predation hypothesis in *Drosophila* is still highly speculative.

Other costs of sexual behaviour are related to mating itself. Partridge and her collaborators have clearly demonstrated in a series of papers that continuous exposure to males significantly reduces the life expectancy of females even when there are no differences in egg production under laboratory conditions (Partridge et al. 1986, 1987, Fowler & Partridge 1989, Partridge & Fowler 1990). This increased mortality is partly caused by events prior to mating (Partridge & Fowler 1990), but mating itself also has a deleterious effect (Fowler & Partridge 1989). It is still uncertain whether these costs will be confronted in nature or whether they are merely an artefact due to the level of exposure to males. The duration of daily male-female interaction is positively correlated with the frequency of remating by females in laboratory experiments (Fuerst et al. 1973, Newport & Gromko 1984), and continuous access to males could thus lead to excess mating. In fact, Hoffman & Harshman (1985) have observed that exposing the females of *D. melanogaster* to males without direct contact may even increase their fecundity.

One possibility is that multiple mating may involve benefits that could not be detected here or were not tested. One possible benefit for the females is said to be related to increased genetic diversity in the progeny (e.g. Richmond & Ehrman 1974). There are several potential theoretical mechanisms by which more diverse offspring may affect maternal fitness (e.g. Wallace 1975, Maynard Smith 1976, Loman et al. 1988), but there is little experimental evidence supporting this consideration. No increased progeny production has been detected in insect experiments specially designed to investigate the effects of single vs. multiple paternity within a progeny (e.g. Arnqvist 1988, van Vianen, pers. comm.), and even among plants, where such experiments are more common, the supporting evidence is scant (Karron & Marshall 1990). The present experiments were not designed to test this hypothesis, and do not allow comparison of the success of the progeny broods of once- and twice-mated females. However, offspring genetic variability varied among the twice-mated females in these experiments. Females that subsequently mated with two genotypes certainly had more variable offspring than those that mated with similar males, but the more variable offspring broods were no larger in either species.

Finally, even though mating may be costly, it may still cost less than rejecting the male (Boorman & Parker 1976, Thorhill & Alcock 1983, Arnqvist 1989). The cost of rejection may not only be a matter of time and energy loss. It has been shown that some *Drosophila* males defend small areas of food and oviposition resources against other males (Jacobs 1960, Hoffman 1987, Hoffman & Cacoyianni 1990). If a female which has already mated is being courted around a suitable feeding or oviposition site, accepting a second mating may be less costly than rejection, because effective rejection may include leaving the site and possible losing a suitable feeding or ovipositing resource.

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