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Environmental Effects on Remating in *Drosophila melanogaster*

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Abstract

The effects of density and food on remating were investigated using *Drosophila melanogaster*. The frequency of remating was unaffected by density for some combinations of fly strains but was reduced at low relative densities for other combinations. Until females had used most of their stored sperm, remating was less likely when food was absent or contact with food was prevented. Food availability had little effect on the incidence of remating once stored sperm were depleted and had no effect on initial virgin mating frequency. This study indicates that environmental factors can have a substantial direct influence on the frequency of remating in populations of *Drosophila melanogaster*.

The evolution of different patterns of animal reproduction is the subject of extensive investigation (Blum and Blum, 1979; Bateson, 1983; Thornhill and Alcock, 1983). There is an increasing emphasis on the role of the environment (Borgia, 1979), which can influence the likelihood of mating through factors such as density and the availability of food resources. The present study assesses the effects of population density and food availability on remating in *Drosophila melanogaster*.

Female multiple mating is an aspect of mating behavior that has received considerable attention, partially because it is intimately associated with patterns of sperm usage (Parker, 1970; Prout and Bundgaard, 1977; Smith, 1984). Multiple mating is common in insects, and females often have a specialized organ, the spermatheca, for the storage of sperm (Parker, 1970). The storage of sperm from more than one male may result in sperm competition, and mechanisms that underlie apparent adaptations resulting from sperm competition are

reviewed in Thornhill and Alcock (1983). Sperm competition is observed in *Drosophila melanogaster* when females mate before most of their stored sperm are depleted (Lefevre and Jonsson, 1962). The objective of this work is to evaluate the opportunity for sperm competition by studying environmental factors that may affect the frequency of multiple mating.

The most extensive studies on insect multiple mating have been conducted with *Drosophila melanogaster*. Progeny analysis of wild-caught *D. melanogaster* females has repeatedly documented the presence of multiple insemination (Milkman and Zeitler, 1974; Richmond, 1976; Stalker, 1976; Gromko et al., 1980; Griffiths et al., 1982; Williams and Strobeck, 1986; Marks et al., 1988). Laboratory studies of multiple mating are even more numerous (Demerec and Kaufman, 1941; Kaufman and Demerec, 1942; Chiang and Hodson, 1950; Lefevre and Jonsson, 1962; Manning, 1962, 1967; Hannah-Alava and Puro, 1964; Minamori and Morihira, 1969; Fuerst et al., 1973; Pulvermacher and Timner, 1977; Gromko and Pyle, 1978; Gromko et al., 1980; Hihara, 1981; Pyle and Gromko, 1981; Gromko and Gerhart, 1984; Gromko et al., 1984b; Newport and Gromko, 1984; Letsinger and Gromko, 1985). Some laboratory studies (Manning, 1962, 1967; Gromko and Pyle, 1978; Gromko, et al., 1984b) have shown that the incidence of remating is correlated with the number of eggs laid or progeny produced by females, which can be used as measures of sperm depletion. Generally, this relationship holds when previously mated females are only periodically given the opportunity to remate, but not when they are held together continuously with males (Newport and Gromko, 1984). Gromko et al. (1 984b) have suggested that the periodic interaction design is more representative of mating behavior of *Drosophila melanogaster* in the field and argue that there is normally little sperm competition, because females use most of their stored sperm before remating.

Environmental factors may affect the frequency of remating. Boorman and Parker (1976) proposed that higher population density could increase the incidence of courtship, resulting in a higher frequency of multiple mating. Marks et al. (1988) estimated the frequency of multiple mating from *Drosophila melanogaster* females collected inside and outside a winery. They found that 61% of the females had mated more than once inside the winery compared to 15% outside. The environments were markedly different, and the density of flies inside the winery was extremely high. Gromko and Gerhart (1984) studied remating in vials with density as a variable and found that remating did not increase with density. They argued that density and resource quality do not have a direct effect on remating but may act indirectly by influencing the number of eggs laid and, therefore, the rate at which stored sperm are depleted.

In this paper, we investigate environ- mental effects on mating in the laboratory. The first part of the study extends the range of densities examined. The remainder consists of a series of experiments which explore the effect of food availability on mating of inseminated females. This study is designed to identify factors affecting the frequency of multiple mating and intensity of sperm competition. Because of the number of experiments performed, we will describe the methods specific to each experiment along with the results under headings for each experiment.

General Materials and Methods: Remating Assay

Various stocks of *Drosophila melanogaster* were used. PC was collected near Putah Creek in November 1983 at the University of California, Davis, Yolo Co., CA. DA was initiated from flies collected in January 1983 inside the D'Agostini winery, Amador Co., CA. Both stocks were derived from at least 75 isofemale lines and were held in mass culture for between six months and 2½ years, until the time of the experiments. A recessive sepia eye-color marker (*se*), was obtained in the late 1970s by inbreeding an isofemale line collected near Camino, Amador Co., CA. The brown-dominant (bw^D) line was obtained from the Mid-America Stock Center at Bowling Green, OH. Both bw^D, as an old laboratory stock, and *se*, by virtue of its inbred origin, are likely to be more genetically homogeneous than are the wild-type stocks. All flies were routinely reared in half-pint bottles with 40 ml of medium at 20°–25°C. The bottles were initiated with 20 females and 20 males, which were removed after two days of egg laying. The medium contained the following ingredients: cornmeal (6.2%), semolina (3.1%), sucrose (3.6%), dextrose (7.1%), agar (1.1%), dead yeast (1.5%), and propionic acid (0.5%). In some cases, granules of live yeast were added to the surface of the medium, and this treatment will be referred to as "yeasted." Flies were examined and sorted under CO_2 anesthesia, except as noted.

Inseminated females for the remating tests were obtained by collecting virgin females and males, which were usually held 1–4 days in bottles with medium but no added live yeast. Flies were mated by placing 100 females and 100 males from the same stock into a bottle with yeasted medium, generally for 24 hours. Females were then separated from males and held in yeasted bottles (where oviposition occurs) until they were used in a remating experiment. The females may have mated more than once or not at all, and it would have been more accurate to obtain inseminated females by direct observation (see below). However, the scale of our experiments precluded this approach.

The assay for remating was essentially the same in all experiments. There were three types of first mating: *se*♀ × *se*♂, PC♀ × PC♂, and DA♀ × DA♂. Inseminated *se* females were combined with either PC or DA males for the remating assay. At the end of a remating period, females were separated from males and placed singly in 8-dram shell vials with 10 ml of medium. Progeny were classified after approximately 2½ weeks at room temperature. If all of the progeny (usually more than 50) had sepia-colored eyes, then it was assumed that the female had not remated. If any wild-type progeny were present, then it was concluded that the female remated at least once. Remating in experiments with inseminated PC and DA females was similarly assessed by confinement with bw^D males. This is a homozygous viable dominant mutation, which does not drastically impair the ability of males to mate.

Any virgin female mating only with the second male would have been scored as remated. To assess the impact of this potential problem we evaluated the incidence of virginity after the first mating period. Virgin female and male *se*, PC, and DA flies were held on medium 2–4 days after they were collected. Approximately 100 males and 100 females of each strain were combined in yeasted bottles for 24 hours. The females were placed singly in vials, and mating was scored by the presence of larvae. The incidence of mated females was 98% for *se*, 98% for PC, and 99% for DA. These experiments were repeated

with *se* and PC virgins held 1–4 days after they were collected. The frequency of mating was 99% for *se* ($N = 100$ females) and 99% for PC ($N = 103$ females). Hence, the estimates of remating reported in this paper were not corrected for the proportion of females failing to mate.

Density and Remating

Methods

We determined the frequency of remating at different densities using three types of first matings: *se*♀ × *se*♂, PC♀ × PC♂, and DA♀ × DA♂. One hundred inseminated females were placed in bottles with medium and transferred to fresh bottles every other day. Once-mated *se* females were held 3½ days before exposure to second males. Once-mated PC and DA females were held 6½ days before exposure to bw^D males. Second males were collected as virgins and held at a density of 100 per bottle on yeasted medium. They were approximately seven days old at the time of the remating assay.

Remating took place in cylindrical white cardboard containers (height 17 cm, diameter 17 cm, volume 3,700 cm^3) covered with gauze. A large petri plate (height 15 mm, diameter 14 cm, area 154 cm^2) filled with medium and brushed with a suspension of live yeast was placed inside on the bottom of each container. Flies were introduced to each container by aspiration through a hole in the gauze.

Each of the six experiments (table 1) compared the frequency of remating at a constant high density (125 flies of each sex per container) with one of two lower densities (either five or 25 flies of each sex per container). For each comparison more containers were used for the low-density treatments (21 or six, respectively) than for the high-density treatment (three) to ensure adequate sample sizes. Treatments were arranged randomly on a table. The 12-hour remating period (10:00 P.M. to 10:00 A.M.), consisted of seven hours of darkness, two hours of early-morning light coming through nearby windows, and three hours of overhead fluorescent light. This 12-hour period included the early-morning peak of reproductive activity (Hardeland, 1972; Bellen and Kiger, 1987).

Table 1. Effect of density on remating by *se* and wild-type (DA and PC) females. Probabilities are from *G* tests of independence ($df = 1$) comparing the number of once-mated and remated females at different densities. Density = the number of each sex in each container; mean number of eggs = the total number of eggs laid during the remating period divided by the total number of females; N = the number of randomly sampled females used to estimate the frequency of remating.

		High-density treatment				Low-density treatment				
Female	Second male	Density	Mean number of eggs	N	% remating	Density	Mean number of eggs	N	% remating	P
se	DA	125	0.139	147	13.6	25	0.020	135	4.4	< 0.01
se	PC	125	0.005	132	16.7	25	0.207	128	5.5	< 0.01
se	PC	125	0.000	112	10.7	5	0.000	96	4.2	< 0.10
DA	bw^D	125	0.267	146	32.2	25	0.160	137	32.1	> 0.10
PC	bw^D	125	0.181	133	20.3	25	0.247	134	17.2	> 0.10
PC	bw^D	125	0.080	105	6.7	5	0.067	95	5.3	> 0.10

After the remating period, flies were held at 5°C until they could be transferred to vials (no more than two hours). This is a temperature at which *D. melanogaster* does not mate (McKenzie, 1975). The containers were transferred to room temperature and covered with a lid through which CO_2 was injected immediately to immobilize the flies. The females were placed singly in vials to determine the frequency of remating. Eggs on the surface of each petri plate were counted to determine whether density indirectly influenced remating by affecting egg production.

Results

The effect of density on the frequency of remating depended on the fly strains used. When inseminated *se* females were confined with wild-type males, there was significantly more remating at high density than low density (table 1). No such effect was apparent when females from the two wild-type stocks were combined with brown-dominant males; higher density did not increase remating. Very few eggs were laid on the plates during the 12-hour remating period (table 1), even though the medium surface occupied most of the bottom of the containers and was covered with yeast. There were no significant differences between high- and low-density treatments for the average number of eggs per female in any of the strains, as indicated by *t* tests.

Presence or Absence of Food and Remating

Methods

In a preliminary experiment, *se* females were kept continuously with *se* males for 48 hours; there was no period of "enforced celibacy." Inseminated females were separated from the first males by aspiration. Ten females and ten virgin PC males of the same age were transferred into each of approximately 130 remating bottles. All bottles contained moist tissue; half were otherwise empty, and half contained 40 ml of yeasted medium into which the tissue was partially inserted. Bottles were arranged randomly on trays, which were placed on shelves at room temperature for a remating period of 48 hours.

Results

Almost all females remated when confined with a second male on food medium, but only one remated when confined without food (table 2). In the two-day remating period, a number of factors could have contributed to the results. Starvation in the absence of food may have suppressed remating. Thus, the following experiments used a shorter remating period. Females in empty bottles laid no eggs and could have remained unreceptive because they did not deplete stored sperm (Gromko and Gerhart, 1984). Consequently, the relationship between productivity, resource availability, and remating was studied.

Table 2. Remating by *se* females in bottles with or without food. The second male was from the wild-type PC stock.

	Number of females with only *se* progeny	Number of females with at least one wild-type offspring	Percentage of females remating
Bottles with moist tissue only	551	1	0.2
Bottles with food and tissue	14	655	97.9

Food Availability, Sperm Depletion, and Remating

Methods

The *se*, PC, and bw^D, stocks were used to assess the relationships among the presence or absence of food, sperm depletion, and remating. At the end of an initial 24-hour homotypic-mating period, PC and *se* females were separated from the males, and aspirated into yeasted bottles (15 per bottle). They were then transferred to fresh bottles every day, until the females were laying only a few fertilized eggs in each bottle. Bottles were kept at room temperature, and the progeny were counted after 2½ weeks to obtain a measure of daily productivity (number of adult progeny emerging in each bottle).

Other bottles of inseminated *se* and PC females established in the preceding manner were used to assay the frequency of remating at various time intervals from the end of the homotypic mating period, employing a remating period of 12 hours. The ends of the remating periods coincided with intervals of 1, 4, 7, 14, 21, or 28 days since the end of the homotypic mating period. Remating with seven-day-old virgin males took place either in empty bottles or in bottles with live yeast on the surface of 40 ml of medium. All bottles had 15 ml of H_2O added to the cotton plug. Fifteen females and fifteen males (*se*♀ × PC or PC♀ × bw^D) were aspirated into each bottle from pooled samples. Bottles were placed on a shaded shelf (32 lux) for 12 hours (10:00 P.M. to 10:00 A.M.) in a room with 6–8 hr of darkness.

An additional experiment tested whether male courtship activity was affected by the presence or absence of food. Inseminated *se* females were transferred daily to fresh bottles as described in the previous experiment. Courtship was observed on two different calendar dates that corresponded to days 4 or 14 after the first mating. Fifteen females and 15 seven-day-old PC males were aspirated into bottles with or without food. Water was added to the cotton plug of all bottles (three to four bottles per treatment). Males and females were synchronously combined an hour before starting the observations (four observation intervals, 40 minutes apart, starting at 10:00 P.M.). At every observation interval, each treatment was observed for five minutes, and the total number of courtship events was recorded. Any of the following constituted a courtship event: pursuit of a female, facial contact with female genitalia, lifting a wing in front of a female, a circling courtship dance, an attempted mount, or mounting of a female.

Results

Figures 1 and 2 present the mean daily productivity per bottle with 15 mated *se* or PC females. Means and standard deviations were based on counts from 15 replicate bottles of each stock. The average total productivity per female was 152.7 for *se* females and 334.7 for

PC females. These values were slight underestimates of the total productivity, because the number of progeny from eggs laid during the 24-hour mating period were not included. Table 3 reports the cumulative percentages of productivity after the initial mating period and the percentages of females remating in bottles with and without medium at various intervals after the initial matings. The percentage of females remating in empty bottles versus bottles with medium was analyzed at each census point by a *G* test. For both *se* and PC females, the incidence of remating was substantially higher in bottles with medium on days 1, 4, 7, and 14 (table 3). Note that, for the significant differences on day 14, the frequencies of remating in empty bottles were much higher than on day 7. Significant differences disappeared at the later time points, as remating frequencies approached 100% for both treatments. There was an association between cumulative productivity and remating frequency. The product-moment correlation coefficients (*r*) were 0.97 and 0.87 for *se* females with and without food, respectively, and 0.95 and 0.93 for PC females with and without food, respectively. While much of the remating in the presence of food occurred well before sperm depletion, most remating in empty bottles occurred considerably later, at high levels of cumulative productivity, presumably when most of the stored sperm were depleted. The delay in remating when no food was present may be due to decreased female receptivity and/or decreased male courtship activity.

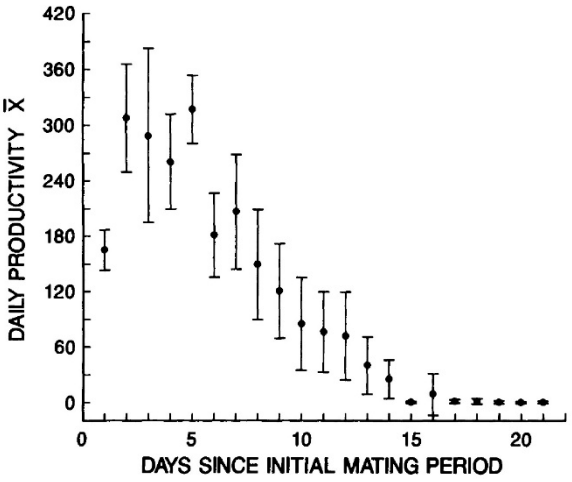

Figure 1. Daily average number of adult progeny emerging per bottle, each with 15 *se* females. Error bars are based on counts from 15 bottles and represent ± one standard deviation.

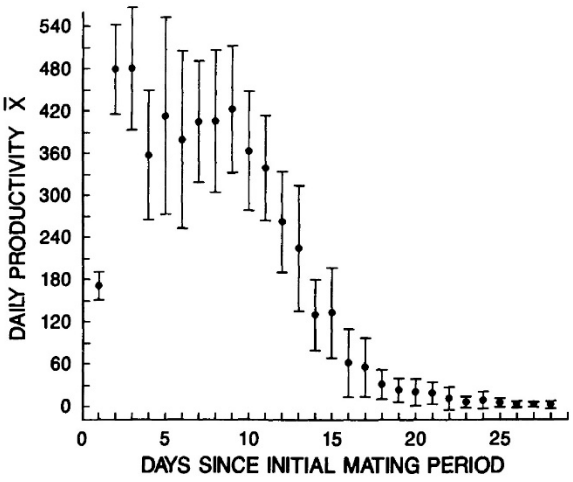

Figure 2. Daily average number of adult progeny emerging per bottle, each with 15 PC females. Error bars are based on counts from 15 bottles and represent ± one standard deviation.

Table 3. Cumulative productivity after the initial 24-hr mating period and periodic census of remating in bottles with and without food medium. Probabilities are from *G* tests of independence comparing the number of once-mated and remated females in bottles with and without food. "Time since first mating" is the period between the end of the initial 24-hr mating period and the end of the 12-hr remating period.

Time since first mating (days)	Cumulative productivity (%)	With food		Without food		
		% remating	*N*	% remating	*N*	*P*
Mated *se*♀ × PC♂						
1	15.0	67.7	69	0.0	66	< 0.0001
4	52.2	89.5	57	4.1	74	< 0.0001
7	76.8	92.5	40	21.4	56	< 0.0001
14	97.7	100.0	39	81.1	37	< 0.0001
21	100.0	100.0	29	95.2	21	> 0.10
Mated PC♀ × *bw*^{D}♂						
1	4.2	22.5	71	0.0	71	< 0.0001
4	26.1	63.9	72	12.0	75	< 0.0001
7	55.3	80.9	72	7.4	81	< 0.0001
14	94.0	98.4	62	79.7	64	< 0.0001
21	99.4	98.6	70	98.3	59	> 0.10
28	100.0	100.0	72	98.1	52	> 0.10

In the experiment to determine the effect of food availability on male courtship, the mean (SD) numbers of courtship events per bottle, averaged over the four observation periods, were: 47.0 (3.41) on medium and 16.0 (1.59) in empty bottles on day 4; and 68.7 (2.15) on medium and 49.3 (2.05) in empty bottles on day 14. A two-way ANOVA on the square-

root-transformed variates revealed a significant food-treatment effect ($P < 0.01$) and day effect ($P < 0.01$) and suggests the presence of a day-by-food interaction effect ($0.10 > P > 0.05$). On day 4, only two matings were observed in the first hour after males and females were combined, and both were in bottles with medium. In the first hour on day 14, 11 matings were observed in empty bottles, and 18 were observed in bottles with medium. Overall, the pattern of male courtship activity with respect to food and day corresponds to the remating pattern.

Food Availability and Virgin Mating

Methods

A related question is whether virgin females, like sperm-depleted females, mate as readily in the absence as in the presence of food. The mating incidence of virgin *se* and PC females with virgin males (PC and *bw*^{D}, respectively) was therefore determined on medium and in empty bottles. Fifty virgin flies of each sex were aspirated into an empty bottle or a bottle with 40 ml of yeasted medium. Each bottle was placed on its side with an inserted aquarium bubbler (a porous cylinder) attached to the end of a hollow needle, which was connected with tubing to a CO_2 supply. When treated gently, copulating pairs remained together under CO_2 anesthesia, so the number of mating flies in a bottle could be determined at the end of a remating period. The aquarium bubbler dispersed gas evenly and prevented the flies from being blown around inside the bottle. Trials were conducted at room temperature under fluorescent ceiling lights. Pilot experiments indicated that mating speed differed for the two mating combinations, so *se* females and PC males were allowed only 10 minutes to mate, while PC females and *bw*^{D} males were allowed 20 minutes. Almost all flies that mated in a 10- or 20-minute interval were detected because copulation typically did not start for at least five minutes after the flies were introduced into a bottle and generally lasted for 15–20 minutes. The experiments were conducted between 7:30 and 10:30 P.M. and between 6:30 and 9:30 A.M. (corresponding to the beginning and end of the remating period in the experiment involving sperm depletion and remating).

Results

The presence of food did not increase the probability of mating (table 4). Paired *t* tests on angular transformations of the data indicated no statistically significant differences. Neither mating of virgin females nor remating of sperm-depleted females were reduced by the absence of food.

Table 4. Mating of virgin females in bottles with and without food medium. Males and females are from the same stocks used in the remating experiments. The mean percentage of females mating is given, with the standard deviation in parentheses.

	Virgin *se*♀ × PC♂		Virgin PC♀ × *bw*^{D}♂	
Treatment	Evening	Morning	Evening	Morning
Without food	48 (3.5)	59 (5.8)	37 (12.0)	47 (3.0)
With food	44 (15.6)	52 (6.0)	31 (8.3)	39 (12.9)

Remating and Environmental Cues

Methods

Since the propensity of inseminated females to mate appeared to depend on the availability of food, the nature of the environmental cues involved was explored. Remating was assayed in "split bottles," which were cut 2–4 cm above the base. The basal portion of half of the bottles was filled with yeasted medium to the level of the cut, and the top portion was attached with tape. Yeasted medium was placed in the basal portion of the other bottles to about 0.5 cm below the cut, and the base was covered with gauze before being attached to the top, so that flies in the top portion would be physically separated from the food but exposed to visual and olfactory cues from it. For the remating assay, 15 inseminated PC females were aspirated into each of the bottles, followed by 15 seven-day-old bw^D males. The females had been separated from the PC males used in the first mating for 3.5 or 6.5 days. Remating was assayed for 12 hours (10:00 P.M. to 10:00 A.M.) thus, the ends of the remating periods corresponded to periods of four or seven days since the first mating period. There were five replicates of each treatment.

Results

Females with access to food were more likely to remate than those without access (table 5). The duration of the remating period was 12 hours, and it seems unlikely that females in contact with food could have laid enough eggs (depleted a sufficient amount of stored sperm) to account for the considerable difference in remating between treatments. Contact with food is apparently related to the high incidence of remating in bottles with medium.

Table 5. Frequency of remating between PC females and bw^D males when flies are allowed contact with food or are separated from it by gauze. Probabilities are from *G* tests of independence comparing the number of once-mated and remated females in the contact and no-contact treatments.

Time since initial mating period (days)	Percentage of females remating			
	Contact	No contact	*N*	*P*
4	26.6	3.0	131	< 0.001
7	75.6	33.3	154	< 0.001

Discussion

In our experiments, higher density was accompanied by increased remating, only in the *se* stock. This stock was probably more genetically uniform than PC or DA, but it also was derived from a natural population. Its mating behavior might or might not be a result of the sepia phenotype. The results indicate that there is genetic variation for density dependence in remating frequency.

The results with wild-type first matings were similar to those of Gromko and Gerhart (1984), in that the frequency of remating did not increase with higher density. Our study may be seen as an extension of the one conducted by Gromko and Gerhart (1984), since

our highest density corresponds approximately to their lowest, regardless of whether densities are calculated on the basis of food surface area, container area, or container volume. Because our densities were relatively low, it is not surprising that we failed to detect the reduction in remating at high density reported by Gromko and Gerhart (1984). The relationship of these experiments to field conditions is a matter of conjecture. In natural populations, densities may often be low, and encounters between individuals may be infrequent. However, flies could be concentrated on resources, and there appears to be a pheromone-mediated aggregation response (Spence et al., 1984; Bartelt et al., 1985; Harshman and Hoffmann, 1987a), which could also contribute to locally high density.

In studies on the relationship between productivity (sperm storage) and remating, Newport and Gromko (1984) have shown that experimental design is an important factor. These authors concluded that, under periodic confinement (exposure to males for two hours or less per day), females remate after their supply of stored sperm is largely depleted, and there is little opportunity for sperm competition; however, under continuous confinement (usually for 24 hours or more), remating occurs before substantial depletion of sperm from the first male, thus providing an opportunity for sperm competition. Perhaps the most direct verification of the association between sperm storage and remating under periodic confinement employed counts of sperm stored (Gilbert, 1981; Gromko et al., 1984a). Also Manning (1962), using the periodic-confinement design, showed that if egg production is suppressed by holding flies on medium without a protein source, then these females are less likely to remate than are females that are allowed to lay eggs and deplete stored sperm.

Numerous studies have employed continuous confinement; the most definitive is that of Bellen and Kiger (1987). They continuously observed vials with three males and one female of the Canton S strain for one week under controlled photoperiod and found a circadian rhythm with a peak of mating in the early-morning hours. In their study, 70% of the matings occurred between 10:00 P.M. and 10:00 A.M. The average female mated once each day.

There are potential problems with both periodic- and continuous-confinement designs. Continuous confinement may restrict female decamping, and the concentration of male stimuli may cause females to mate at an unusually high frequency (Newport and Gromko, 1984). However, Partridge et al. (1987) found that female decamping did not usually terminate courtship by *D. melanogaster* males in the field. Another potential problem arises from the early-morning peak of mating, which has been documented in the laboratory (Hardeland, 1972; Bellen and Kiger, 1987) and in the field (Taylor, pers. comm.; Partridge et al., 1987). This could be the only time the sexes naturally come together. The continuous-confinement method could be artificial for this reason. A problem with periodic-confinement experiments in any of the published remating studies is that the two-hour observation period does not correspond with the early-morning peak of mating activity. This could result in a substantial underestimation of remating. Handling of flies prior to the remating assay could also reduce the probability of remating. However, Harshman and Hoffmann (1987b) compared a passive method of combining males and females with active transfer and found no difference in remating frequency. Our remating assay differs from the usual periodic design in that the flies were held together longer and the remating interval encompassed the early-morning predawn peak of reproductive activity.

In our study, a relatively high frequency of remating on food medium occurred at low levels of cumulative productivity (table 3), which indicates that there is an opportunity for sperm competition in most of these females. The degree to which productivity was underestimated is unlikely to alter this conclusion, because only progeny from the day of the first mating were missed and egg production typically does not peak for several days after mating. There was a dramatic difference in remating frequency in bottles with and without food (tables 2 and 3). Remating in empty bottles remains infrequent long after it is common in food bottles (table 3). Thus, remating is not only a function of cumulative productivity (sperm depletion) but also depends directly on the environment. Other laboratory studies have documented that environmental factors, such as CO_2 and ether, affect remating (Gromko et al., 1984a). Our results may be more important for understanding the behavior of *D. melanogaster*, because access to food is a variable that is relevant to the natural ecology of the flies.

The observations of male courtship activity suggest that the higher incidence of remating on food may be mediated by males, females, or both. The decrease in remating in the absence of food could be due simply to reduced courtship by males, or the reduced courtship by males could be due to lower attractiveness of females.

There are many factors that could give rise to variable results when different experiments in this study are compared. For instance, the differences in remating frequency described in tables 1 and 3 may be due to the different physical conditions in the two sets of experiments.

Letsinger and Gromko (1985) have proposed that females become receptive to remating when a low-threshold number of sperm remain in storage. In the present study, the level of cumulative productivity attained before most females remated in bottles without food was similar to the threshold for remating (79–90% cumulative productivity) estimated from the periodic-confinement studies of Gromko and Pyle (1978) and Gromko et al. (1984b). As shown in table 3, the incidence of remating of the *se* females in bottles without food increased from 20% to 80% between days 7 and 14, as the cumulative productivity increased from 77% to 90%. The same pattern was observed for PC females: remating in the bottles without food increased from 10% to 80% as cumulative productivity rose from 55% to 94%. Thus, it is possible that the lack of food in our study and periodic confinement in previous studies may both inhibit remating. Our study suggests that contact with food raises the postulated threshold (Letsinger and Gromko, 1985) below which the number of stored sperm must fall before remating occurs. Therefore, environmental variables may influence the incidence of remating directly (rather than just determining how many eggs have been laid and how many sperm remain in storage).

The decaying fruits used as resources by *D. melanogaster* are ephemeral (Nielsen and Hoffmann, 1985). The dramatic effect of food availability may be a manifestation of a natural response to the variation in resource availability experienced by flies in the field. This may help to explain the variation in levels of multiple insemination observed in different field studies. It is significant that differences in the frequencies of multiple inseminations were found in the one study in which two contrasting habitats were sampled (Marks et al., 1988). It seems clear that the generally high level of multiple insemination observed in the

field provides ample opportunity for sperm competition between different male genotypes, and we believe we have identified one of the factors that enhances the opportunity for sperm competition.

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