Work in progress however appears to indicate that very late-hatched larvae are more genetically heterogenous and can be of 2 or more different genotypes and that complementary interaction between 2 late-hatched genotypes may give faster-hatching progeny. In all 4 populations, a proportion of F_1 larvae which would have hatched only in exudate when young, hatched readily in water at an older age. This suggests that the gene or genes which cause hatching in response to exudate are active during diapause and given time will produce enough product to allow hatching to proceed in the absence of the hatching factor. Accordingly, some of the larvae which hatch in water from unselected old populations of *H. rostochiensis* may well belong to this category and this may explain why in earlier work[6], matings of water-hatched parents gave F_1 which largely segregated for exudate-hatched phenotype.

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Repeated mating by female *Drosophila melanogaster*: The adaptive importance

D.W. Pyle[1,2] and M.H. Gromko

Department of Zoology, Indiana University, Bloomington (Indiana 47401, USA), 14 September 1977

Summary. Comparisons of productivity, fertility and fecundity between once-mated females of *D. melanogaster* and females given the opportunity to remate reveals that remating sustains high levels of these fitness traits.

Females of many insect species mate numerous times throughout their reproductive lives and often store large numbers of sperm. The storage of sperm would seem to reduce or perhaps eliminate the necessity to remate. However, both laboratory and field studies of *Drosophila*[3-7] reveal that remating is common. But there is still no clear explanation of the adaptive significance of remating for female *Drosophila*. In fact, Ikeda[8] demonstrated that multiple copulation in *D. mercatorum* has a deleterious effect on fitness.

A number of hypotheses have been advanced to explain the origin and maintenance of remating behavior by female *Drosophila*. Remating could result in increasing the genetic heterogeneity of a female's offspring. Richmond and Ehrman[3] postulated that it is advantageous for a female to remate and produce eggs of multiple male parentage since larval competition is reduced in genetically heterogenous culture bottles[9]. Anderson[4] suggested that females need to replenish their sperm supply after its depletion due to egg laying. Remating would also be favored if the first insemination were inadequate or if the energy cost to the female were greater in rejecting a persistant male than in remating[10]. Finally, competition between successive mates for the fertilization of a female's eggs could result in male mating strategies which influence female remating[11-13]. In this paper we explore the relationship between the timing of successive matings by female *D. melanogaster* and their fitness as measured by productivity (the number of progeny produced per female), fecundity (the number of eggs laid per female) and fertility (the proportion of eggs laid which hatched).

Methods and materials. The flies used in this study were taken from a 4-year-old wild-type cage population synthesized from strains of heterogeneous origin[14]. The procedure for obtaining once-mated females used throughout this study is as follows. Virgin females, 3 days old, were put individually into 8 dram food vials with 2 males aged 3-5 days. Vials were examined at 10-min intervals and those containing copulating pairs were gently set aside (copulation in *D. melanogaster* lasts approximately 20 min[15]). Males were removed with an aspirator within 30 min of the completion of copulation.

Single-mated females remained in the yeasted food vials and were treated in one of 2 ways. Females in treatment group 1 (n = 107) were simply transferred without etherization into fresh food vials daily for 14 days and twice more at 4-day intervals. The number of progeny produced per single-mated female per day was recorded. Females in treatment group 2 (n = 70) were subcultured daily as above for 22 days but these females were given the opportunity to remate. 2 males were placed with each female for 2 h each day for 15 days and copulations were scored visually as in the first matings. These group 2 females were transferred into fresh food vials for the last 8 days without the opportunity to remate. The number of progeny produced per female per day was scored and compared to the productivity of single-mated females. Note that group 2 females may include single-mated females as well as those that remated a number of times.

Results and discussion. The productivity of group 2 females was much greater than group 1 females (figure 1). Females from both groups had similar productivity for the first 5 days of testing but thereafter the number of progeny produced by single-mated females per day dropped rapidly approaching 0 at the end of the study, at which time dissection revealed that these females were devoid of sperm. The productivity of group 2 females remained high during the 2 weeks when males were provided, after which their productivity dropped as in group 1 females. The mean number of progeny per female following only 1 mating was 528 and the maximum number of offspring produced by

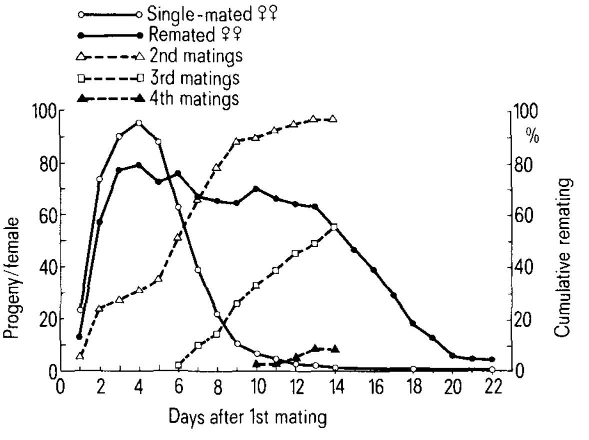

Fig. 1. The productivity (number of progeny produced per female on the left ordinate) in 2 groups of female *D. melanogaster* which are either permitted to mate only once (open circles) or given the opportunity to remate (closed circles) over a period of 22 days (abscissa). The right ordinate shows the cumulative percentage of remating in females given the opportunity to remate (open triangles, double-matings; squares, triple-matings; closed triangles, quadruple-matings).

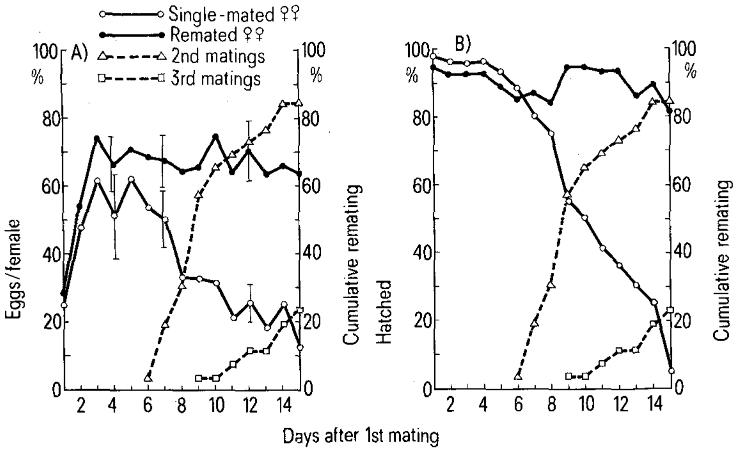

Fig. 2. *a* The fecundity (number of eggs laid per female on the left ordinate) in 2 groups of female *D. melanogaster* which were either allowed to mate only once (open circles) or were given the opportunity to remate (closed circles) over a period of 15 days (abscissa). The right ordinate shows the cumulative percentage of remating in females given the opportunity to remate (open triangles, double-matings; squares, triple-matings). *b* The fertility (proportion of eggs laid which hatched on the left ordinate) in 2 groups of females which were either allowed to mate only once (open circles) or were given the opportunity to remate (closed circles) over a period of 15 days (abscissa). The right ordinate shows the cumulative percentage of remating in females given the opportunity to remate (open triangles, double-matings; squares, triple-matings).

1 female was 776. All single-mated group 1 females were fertilized following 1 copulation with 78% producing over 450 progeny. The mean number of progeny per group 2 females was 938 for the 2-week period when males were present daily and 1053 over the entire 22-day testing period. Of the 70 group 2 females 97% double-mated, 56% triple-mated and 9% mated 4 times (figure 1). The average time from 1st to 2nd mating (5.9 days) corresponds to a time period in which single-mated females had produced 82.6% of their total progeny, indicating that remating occurs before the sperm supply is exhausted. This conclusion was verified in a separate experiment by disrupting 15 remating pairs within 2 min of the initiation of copulation. This is insufficient time for new sperm to be transferred[16]. The females were dissected and in all cases sperm was present in their storage organs.

We suspected that the dissimilarity in peak productivity between group 1 and group 2 females (figure 1) was within the normal range of variability for productivity and we confirmed this in 2 ways. First, while repeating the 1st 6 days of treatment 2 conditions (to disrupt copulating pairs) the productivity was again measured and found to be slightly greater than the productivity of group 1 females tested previously. Secondly, we repeated the experiment in a modified way, measuring fecundity and fertility coordinately with the timing of remating.

Fecundity and fertility were compared between 26 single-mated females and 26 females given the chance to remate daily as in the productivity study. In this case the food medium was specially prepared for ease of egg counting. Plastic spoons with a capacity of approximately 6 ml were filled with corn meal-agar-molasses medium to which charcoal was added. 1 drop of yeast was brushed over the food before these spoons were placed in large vials with individual females. The spoons were changed daily for 14 days for both groups of females. Group 1 females were not given the opportunity to remate whereas group 2 females were supplied with 2 males for 2 h each day at the time of transfer. All matings were recorded. The eggs laid in each 24-h period were counted and after an additional 36 h the hatched eggs were counted giving measures of fecundity and fertility, respectively.

As in the productivity study, female fecundity and fertility remained at high levels in group 2 females, whereas both measures of fitness began to decrease in group 1 females 7 days after the 1st mating (figure 2). The rate of egg laying was reduced in once-mated females as their sperm store was depleted (figure 2, a), however, this decrease was not sufficient to prevent an increasing percentage of unfertilized eggs from being laid (figure 2, b). The mean number of eggs which hatched following single-mating was 416 and the mean number of eggs which hatched from group 2 females was 869.

Again a high degree of remating was observed, with 84% of the group 2 females mating twice and 23% of them mating 3 times. The average time from 1st to 2nd mating (9.3 days) represents a 3.4-day increase over the average time to remating in the productivity study. In the 9-day period, single-mated females produced 88.7% of their total progeny, indicating again that females given the opportunity to remate do so before they have exhausted their stored sperm. Therefore, remating is not time dependent but is correlated with the rate of progeny production and sperm utilization.

Previous studies[10,16-19] failed to show differences in productivity and fecundity between single-mated females and double-mated females. Elsewhere[13] we argue that this is related to the use of experimental techniques which deemphasize the role of the female in determining the timing of remating. The results presented here are sufficient to describe the adaptive significance of remating and the timing of remating as they effect the fitness of female *D. melanogaster*, at least under our laboratory conditions. Females remate when their productivity (figure 1), fertility and fecundity (figure 2) begin to decrease as a result of the depletion of stored sperm. The net result of remating is to refill the females' sperm storage organs and thereby maintain high levels of these fitness parameters.

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2 Present address: Biology Department, Skidmore College, Saratoga Springs, New York 12866, USA.

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