ASSOCIATION BETWEEN MATING SPEED AND FERTILITY IN *DROSOPHILA ROBUSTA*[1]

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THAT genic determination within naturally occurring karyotypes has influence on the mating speed has been shown by SPIESS and LANGER (1961, 1964a,b) in *Drosophila persimilis* and in *D. pseudoobscura*, by KAUL and PARSONS (1965) in *D. pseudoobscura*, by BRNCIC and KOREF-SANTIBAÑEZ (1964) in *D. pavani*, and by PRAKASH (1965, 1967) in *D. robusta*. In order to understand fully the relative importance of mating speed as a component of fitness of karyotypes, it is important to know whether fast mating and slow mating genotypes differ only in the speed with which they achieve their first mating or in that fast mating genotypes are also endowed with a greater capacity to mate repeatedly in a unit time, thereby producing more offspring. A positive correlation between fast mating, repeat mating, and number of offspring produced per unit time would mean that mating speed is a very important and easy-to-measure indicator of fertility. Experiments were performed to see if a positive correlation exists between fast mating and fertility in *D. robusta*.

METHODS

The flies for the experiments were obtained by interstrain and interkaryotype crosses of the following selected homokaryotypic strains: four strains, A_1, A_2, A_3, and A_4, of $\frac{XL\ XR\ 2L\ 2R\ 3R}{XL\ XR\ 2L\ 2R\ 3R}$ karyotype; and four strains, B_1, B_2, B_3, and B_4, of $\frac{XL\ XR\text{–}1\ 2L\ 2R\ 3R}{XL\ XR\text{–}1\ 2L\ 2R\ 3R}$ karyotype. Each strain was started from a single-pair mating of homokaryotype flies caught at Creve Coeur, Missouri, in June 1965. The two parent flies in each case had their karyotypes determined by first being crossed to Standard flies. The wild females used as founders were therefore deseminated twice before being mated to a chosen male. Four groups of flies, all of karyotype $\frac{XL\ XR\ 2L\ 2R\ 3R}{XL\ XR\ 2L\ 2R\ 3R}$ were obtained for experimental use by crossing 10- to 15-day-old flies in the manner $A_1 \times A_2$, $A_2 \times A_3$, $A_3 \times A_4$, and $A_4 \times A_1$. Similarly, four groups of $\frac{XL\ XR\text{–}1\ 2L\ 2R\ 3R}{XL\ XR\text{–}1\ 2L\ 2R\ 3R}$ were obtained by interstrain crosses of the type $B_1 \times B_2$, $B_2 \times B_3$, $B_3 \times B_4$, and $B_4 \times B_1$.

Four groups of heterokaryotype females—$\frac{XL\ XR\quad 2L\ 2R\ 3R}{XL\ XR\text{–}1\ 2L\ 2R\ 3R}$—were founded by interkaryotype crosses of $A_1 \times B_1$, $A_2 \times B_2$, $A_3 \times B_3$, and $A_4 \times B_4$. Approximately 20 pairs of virgin flies were used as parents for each experimental group. Flies were subcultured to fresh food-bottles every four days. Developing larvae were heavily yeasted. Newly emerged flies were collected and sexed every 24 hours. Twelve flies of one sex were left in a well yeasted fresh food vial for a period of three days and were changed to fresh food every third day. Mating experiments were

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done with 12- to 15-day-old virgin flies. One male of the karyotype $\frac{XL\ XR\ 2L\ 2R\ 3R}{XL\ XR\ 2L\ 2R\ 3R}$ or $\frac{XL\ XR\text{–}1\ 2L\ 2R\ 3R}{XL\ XR\text{–}1\ 2L\ 2R\ 3R}$ was given the choice of mating with three marked females, one each of three different karyotypes, for a period of two hours. Since the age of the flies varied from 12 to 15 days, care was taken to test equal numbers of males of two different karyotypes at the same age in order to make the results comparable. The $\frac{XL\ XR\ 2L\ 2R\ 3R}{XL\ XR\ 2L\ 2R\ 3R}\left(\frac{XR}{XR}\right)$, $\frac{XL\ XR\text{-}1\ 2L\ 2R\ 3R}{XL\ XR\text{-}1\ 2L\ 2R\ 3R}\left(\frac{XR\text{-}1}{XR\text{-}1}\right)$, and $\frac{XL\ XR\ \ 2L\ 2R\ 3R}{XL\ XR\text{-}1\ 2L\ 2R\ 3R}\left(\frac{XR\ \ }{XR\text{-}1}\right)$ females were marked by making a small circular hole near the tip of the submarginal cell of the left, right, and both wings, respectively. The females were etherized at the time of marking, which was done 3 to 4 days before the mating observations were performed. The flies were not etherized again after this period. At the time of the experiment, three marked virgin females were transferred to a well yeasted fresh food vial by aspiration. The food vial had been equilibrated with the room temperature before being used as a "mating vial." The females were given 5 to 10 minutes to overcome aspiration "shock" and then one healthy male was taken and left in the "harem" for 2 hours. The male was always taken from a stock different from those of the females in his "harem." This was done to avoid any possible effects which might result from rearing the males and females in the same bottle. All four flies in the mating vial were of the same age. The mating vials were spread out, 20 in a single row, on a raised platform so that they were level with the observer's eyes. A 15w fluorescent light situated 12 inches from the vials was used in addition to the regular overhead lights, in order to make the flies more visible. Only 20 mating-vials were observed at a time because the average duration of copulation in this species is 37 seconds, and thus the chances of missing a mating increase with increasing number of vials. A stop-watch was started just after the male was placed in the harem. A magnifier lens (×7) was used to distinguish the marks on the female engaging in a copulation. The experiments were run between the hours of 7 to 10 PM in November 1965.

The flies were raised and the experiments were carried out at 25° ± 1°C. After a 2-hour period, the male was aspirated out of the mating vial and the mated females were placed individually in well yeasted fresh food-vials and subcultured every two days through three changes. In some cases, the female was subcultured an additional time if she was still laying fertilized eggs. The developing larvae in the cultures were well fed with yeast suspended in water. When third instar larvae began appearing, folded Kleenex tissue paper was placed in each vial to provide a dry surface for the larvae to pupate. It must be said that there were some deaths due to larval and pupal mortality. No account, however, was kept of such deaths. The emerging flies were counted and only when it was absolutely certain that no more flies would emerge were the cultures discarded. These counts provide a measure of the reproductive success of the mating events which occurred during the observation period.

RESULTS

Since no differences could be detected in the mating activity of males and females of different karyotypes, the whole data have been treated as a homogeneous sample without regard to the karyotype of the flies.

Association between first mating and repeat mating in males: Table 1, Column 5, presents the total number of repeat matings accomplished by males of different groups. The groups are arbitrary classifications based on the speed with which the males perform their first mating. Proportionately more repeat matings are performed by the fast-mating males as compared to the slow (see column 6, Table 1). Nevertheless, the argument can be raised that the number of repeat

TABLE 1

Association between fast mating, repeat mating, and fertility (number of offspring produced) in the males

1	2	3	4	5	6	7	8	9
Group	Time of first mating (min)	Number of first matings	Number of repeat matings in first 10 min	Total number of repeat matings	Ratio of repeat/ first matings	Average number of offspring produced	Average number of offspring produced by 1st female	Average number of offspring produced by 2nd female
1	0.00–20.00	60* (75.12)	24† (26.09%)	103* (87.88)	1.72	211.48‡ (N=77)	125.45 ± 7.53§ (N=77)	107.18 ± 7.09‖ (N=52)
2	20.01–40.00	31* (36.41)		48* (42.59)	1.55			
3	40.01–80.00	45* (34.56)	4† (5.9%)	30* (40.44)	0.67	142.86‡ (N=50)	112.72 ± 7.57§ (N=50)	91.27 ± 8.82‖ (N=15)
4	80.01–120.00	23* (12.90)		5* (15.10)	0.22			

* $\chi^2_{(3)}=27.6$ ($P < 0.001$). Expected numbers of matings (in parentheses) were calculated from the marginal totals of a 4×2 contingency table with 3 df.
† $\chi^2_{(1)}=7.0$ ($P < 0.01$ with Yates' correction).
‡ $t_{(125)}=3.9$ ($P < 0.001$).
§ $t_{(125)}=1.15$ ($0.2 < P < 0.3$). Not significant.
‖ $t_{(65)}=1.14$ ($0.2 < P < 0.3$). Not significant.

matings is greater in groups 1 and 2 simply because the male has more time left after the first mating for repeat matings than do the males of groups 3 and 4.

Column 4 of Table 1, however, shows that the percentage frequency of repeat matings in the 10 minutes subsequent to the first mating is significantly greater in the males which complete their first mating in the 0.00–40.00 minute interval than in those which initiate their first mating after 40.00 minutes. The period of 10 minutes after the first mating was chosen because larger numbers of first matings of group 4 could be included. It therefore seems that fast-mating males in *D. robusta* have a greater capacity to perform more matings per unit time as opposed to the slow.

Association between fast mating and repeat mating in females: Table 2 presents the data of the frequency of repeat mating in females. The females are called first, second, or third female depending on the order in which the female mated with the male; for example, there were 157 females which participated in the male's first sexual act. These 157 first females engaged in 50 additional matings after the first mating. It can be seen that the frequency of repeat mating is more than expected on the basis of random binomial proportions in the first female than in the second or the third female (Column 3 of Table 2). In order to distinguish between the possibilities that the first females are involved in more repeat matings because they have more time left after the first mating, or because they have a greater capacity to repeat-mate per unit time, the data were analyzed in the following manner. Suppose that of the three females in the mating vial, one is the first mater. The random chance that either one of these three females will be the second choice of the male is ⅓. Then, are more than ⅓ of the first-mater females involved in the second mating of the males? Section A of Table 3 shows that out of 98 first-mater females, only 23 were included in the second mating of the male. This is significantly less than the expected ⅓ which is ⅓ × 98 = 32.7 ($\chi^2_{(1)} = 4.3$, $P < 0.05$). Likewise, the second females seem to be engaged in a smaller number than expected (though not significantly) of third matings of the male (Section C of Table 3). However, the number of first females that

TABLE 2

Association between fast mating and the number of repeat matings in the females

1 Serial order in which females mated with the males	2 Number of first mating	3 Total number of repeat matings	4 Proportion of repeat matings per first mating
First female	157	50	0.32
	(165.48)	(41.52)	
Second female	86	17	0.20
	(82.34)	(20.66)	
Third female	24	0	0.00
	(19.18)	(4.82)	
	$\chi^2_{(2)} = 9.0$	$P < 0.015$	

Expected numbers of matings (in parentheses) were calculated from the marginal totals of a 3×2 contingency table with 2df.

TABLE 3

Association between initial mating and repeat matings in females

A. Least number of times male mated	Total number of first matings	Total number of second matings by the first female	Expected number of second matings on the basis of randomness	$\chi^2_{(1)}$	P
2	98	23	32.7	4.3	< 0.05
B. Least number of times male mated	**Total number of first matings**	**Total number of third matings by the first female**	**Expected number of third matings on the basis of randomness**	$\chi^2_{(1)}$	P
3	58	21	19.33	0.2	> 0.50
C. Least number of times male mated	**Total number of second matings**	**Total number of third matings by the second female**	**Expected number of third matings on the basis of randomness**	$\chi^2_{(1)}$	P
3	58	16	19.33	0.9	> 0.25

took part in the third mating is a little more than expected (Section B of Table 3). These differences are not significant.

It appears, then, that the females show a disinclination to mate again for a certain refractory period, and that the greater proportion of repeat matings in the first female is due to the longer time available in which to engage in repeat mating.

Correlation between mating activity and fertility of females: Section A of Table 4 shows that remating does not increase the productivity of the females in terms of the offspring produced. Since remating does not increase the productivity of the females, no distinction was made between once- or twice-mated females and the data were classified according to the order with which females mated with the male, that is, first, second, or third female. It can be seen from Section B of Table 4 that the number of offspring produced by the second female is significantly less than that produced by the first female. Section C of Table 4 presents the number of offspring produced by the first, second, and third female when a single male mates with three different females in that order. It can be seen that the productivity of the males declines progressively in successive matings. The nonsignificance of the correlation coefficient is most probably due to small sample-sizes, because with larger sample-sizes, as in Section B of Table 4, the productivity of the second female is definitely lower than that of the first female.

Correlation between mating activity and fertility of males: Section D of Table 4 shows the productivities of males mating with one female only, two females, or three females. The productivity of males which mate with three females is highest and of those mating with one female only is lowest. There is a highly significant positive correlation between the number of females a male mates with and the number of offspring produced ($r = 0.62$, $df = 122$, $P = < 0.001$).

Since there is a positive correlation between the number of females insemi-

TABLE 4

Association between mating activity and offspring production in females

A. Number of offspring produced as a result of single mating and two matings of the first female

	Average number of offspring produced by the first female which mated once	Average number of offspring produced by the first female which mated twice
Number of offspring	118.90 ± 6.17	121.03 ± 11.31
Number of females tested	90	37

B. Number of offspring produced by the first female and the second female. Both females mated with the same male

Number of males tested	Average number of offspring produced by the first female	Average number of offspring produced by the second female
67	123.27 ± 7.59	100.80 ± 6.16
t for paired comparisons = 2.4	df = 66	$P < 0.02$

C. Number of offspring produced by the first, second, and the third female. All three females mated with the same male

Number of males tested	Average number of offspring produced by the first female (1)	Average number of offspring produced by the second female (2)	Average number of offspring produced by the third female (3)
18	119.72 ± 13.16	93.67 ± 9.02	83.05 ± 13.05
	$r_{1,3} = -0.39$	$r_{1,2} = -0.41$	
	$P \simeq 0.10$	$P = 0.10$	

D. Average number of offspring produced as a result of matings of one female with one male, two females with one male, and three females with one male

	One male mating with:		
	One female	Two females	Three females
Number of offspring	124.22 ± 9.15	224.23 ± 13.46	296.44 ± 16.89
Number of males tested	59	47	18

nated by a male and his productivity, it would be expected that fast-mating males, by virtue of their greater capacity for repeat mating, will have a higher productivity than the slow. Column 7 of Table 1 demonstrates this point. The number of offspring born to fast-mating males is significantly higher ($P < 0.001$) than that of slow maters. Column 8 of Table 1 shows that there is no significant difference in the average number of offspring produced by the first female mates of the fast and the slow males. Neither is there any difference in the number of offspring produced by the second female mates of the fast and the slow males (see Column 9 of Table 1). This, then, indicates that probably there is no difference in the number of sperm deposited by the fast and the slow males in each insemination. The higher fertility of the fast males is then due to their greater capacity to mate repeatedly.

DISCUSSION

Males with faster mating speed would produce more offspring because of their greater inherent capacity to mate repeatedly, which results in the insemination

of more females. Fast mating in males then, provides them with two distinct advantages, (1) the faster the mating speed of the male, the sooner are the females inseminated and the sooner do the offspring develop compared to the offspring of slow mating males. (2) As has been shown in this paper, fast mating males have a higher fertility because of their greater capacity to mate repeatedly. These studies are in agreement with those of FULKER (1966) who found that the fast-mating males in *D. melanogaster* perform larger numbers of copulations and produce more offspring than the slow.

The fast females, however, do not show greater repeat-mating than the slow. Repeat-mating in *D. robusta* females does not lead to an increase in the production of offspring. LEFEVRE and JONSSON (1962) have shown that double mating in *D. melanogaster* females leads to a displacement of sperms deposited by the first mating and a slight increase in the females' productivity (about 15%). However, as these authors point out, the slight increase in the productivity of *D. melanogaster* females as a result of repeat-mating can be accounted for because of the time interval between matings, so that the female has utilized some of the sperms deposited in her by the first mating and has thereby created some space for deposition of additional sperm by the second mating. On the other hand, in *D. robusta*, because of a short time lapse between matings, the female does not have much time to deposit fertile eggs and thereby make room available for the sperm of the next mating. The lack of increase in the productivity of *D. robusta* females as a result of repeat matings is not due to low fecundity since at maturity these females lay, on the average, 100 eggs per day (unpublished records of Professor H. D. STALKER). It is very likely that the *D. robusta* male fills most of the female sperm storage organs by his first mating, as in *D. melanogaster* (LEFEVRE and JONSSON 1962). Fast mating will provide the female with an advantage of receiving the largest number of sperm since the male deposits the largest number of sperm in the first female. Fast-mating genotypes will have greater fitness than slow ones irrespective of the structure of the mating cohort of the species, since fast maters exhibit their advantages both in the presence and absence of competition (PRAKASH 1966).

Selection experiments of MANNING (1961) show that variability exists for the genes which affect male mating speed in *D. melanogaster*. Since male mating speed directly bears on fitness one would expect a strong directional selection in natural populations for the genes for high mating speed. What is the mechanism which maintains the stability of the gene frequencies and therefore maintains the mean mating-speed at a constant value in natural populations? Some possible explanations are listed here.

1. Heterozygotes for the genes which affect male mating speed are over-dominant. This would ensure both the maintenance of genetic variability and the mean mating speed at a stable value.

2. The mean mating speed in the population is maintained at the stable value by counterselection in some other component of fitness. The negative correlation between mating speed and some other fitness character could be such that an intermediate phenotype has the higher fitness (ROBERTSON 1955). It has been

shown by MANNING (1961) in *D. melanogaster* that flies selected for fast mating speed have lower general locomotor activity, while flies selected for slow mating are very active. The genetic variability might be maintained either by overdominance, so that the heterozygotes have the highest fitness or by a balance between mutation and selection.

3. If the heterozygotes are not overdominant, then the stability of the gene frequencies and the mean mating speed in a population might be maintained by recurrent mutations. In view of the strong selection favoring high mating speed, it seems unlikely that recurrent mutations can hold the mean speed at the stable value, unless one assumes that only mutations which lower the mating speed are occurring.

4. The stability of the genetic variability and the mean mating speed is maintained by frequency-dependent selection on the mating speed. As the frequency of high-mating-speed genotypes increases, their mating speed decreases, and vice versa. Such frequency-dependent selections for mating activity have been demonstrated in *D. pseudoobscura* karyotypes (EHRMAN *et al.* 1965).

The question of the maintenance of genetic equilibrium in natural populations for any component of fitness still remains unanswered and obviously is in need of more experimental work.

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SUMMARY

Both sexes in *D. robusta* repeat-mate very frequently. In males, there is a positive correlation between fast mating, repeat mating, and fertility. Repeat mating in females does not increase the productivity. However, fast-mating females receive the largest number of sperm, since the male deposits the largest number of sperm in the first female.

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