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The Aging Effect on Male Mating Activity in *Drosophila melanogaster*

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Noncompetitive mating activity for young (3-day) and old (28-day) Drosophila melanogaster *males was measured under chromosomally homozygous and heterozygous conditions. Old males were consistently less active than young ones under both conditions. Three of 29 homozygous lines exhibited sterility due to aging. Virility at the old age did not correlate with that at the young age. Differences among homozygous lines were highly significant for old and young males, indicating a genetic basis for the trait. Individual variation in "old" virility was shown to be much higher than that in young males.*

KEY WORDS: mating activity; aging; selection: *Drosophila melanogaster.*

INTRODUCTION

It is of fundamental significance to extend our understanding of the quantitative genetic basis of natural variation in fitness components. In estimating the net fitness differentials in *Drosophila*, it has become apparent that the male reproductive component of fitness, virility, may play a more important role than fitness variables in preadult stages and female fertility (Sved and Ayala, 1970; Sved, 1971; Prout, 1971a, b; Bundgaard and Christiansen, 1972; Anderson, 1979; Petit *et al.*, 1980; Brittnacher, 1981; Sharp, 1982; Kosuda, 1983). *Drosophila* males can mate with a considerable number of females within a limited period. *Drosophila melanogaster*, for example, can copulate with more than 10 females per day (Mossige, 1955; Kosuda, 1983). Duncan (1930) reported that one *D. melanogaster* male mated as many as 121 times during his life span of 72

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days. Repeated mating by females is much less frequent. Some males are excluded from a reproductive population, if multiple mating is taken into consideration. Multiple mating contributes to sexual selection and makes the sex ratio in reproduction deviate from 1:1. It also reduces the effective size of a population.

Males with a low sexual vigor, such as very old ones, may be eliminated from a reproductive population by sexual selection. Since the mating activity of the aged individuals which have passed through the reproductive period is not subjected to selection, it is expected that the genetic variability in virility is much higher at an old age than at a young age. It is also expected that the virility reduction in old males is larger in homozygotes than in heterozygotes.

MATERIALS AND METHODS

Male mating activity under chromosomally homozygous and heterozygous conditions was measured in Series A and B, respectively. Series A used 29 nonlethal, nonsterile lines homozygous for the second chromosome. Chromosomes were extracted from a natural population in Katsunuma, Yamanashi, Japan, in the autumn of 1980, by the standard marked inversion method employing the SM5 balancer chromosome. Heterozygous males used in Series B were wild-type progenies from the same natural population. Experimental males were divided into two age groups referred to as "old" and "young" males. Old males were 28 days posteclosion, and young males were 3 days posteclosion.

Males were placed singly into a 3 × 8-cm mating vial on food containing 12 virgin females of a standard laboratory strain designated "2SG," without anesthetization. 2SG females were always 3 to 4 days old. They were maintained at 25°C. After 24 h, 10 of 12 females were randomly chosen for sperm inspection. Male mating activity was defined as the number of females inseminated by single males within 24 h. Twelve replicates each were made for 29 homozygous lines in Series A.

RESULTS AND DISCUSSION

Table I shows the mating activity for "old" and "young" males in homozygous lines. The mating activity of old males over the array of 29 lines was 1.85 ± 0.25, whereas the mean activity of young males was calculated as 5.35 ± 0.31. The difference between old and young homozygous males is highly significant ($P < 0.001$). The virility of old males is consistently lower than that of young ones. The coefficient of variation (SD/mean) in old males (120%) is 2.4 times larger than that in young males

Table I. Mating Activities (Mean ± SE) for Both "Old" and "Young" Males in 29 Chromosomally Hymozygous Lines[a]

Line	Old	Young
KN001	1.92 ± 0.45	5.67 ± 0.85
KN002	1.92 ± 0.58	3.25 ± 0.78
KN005	0	2.75 ± 0.68
KN006	2.58 ± 0.50	5.17 ± 0.72
KN017	0.58 ± 0.23	7.08 ± 0.51
KN020	0	4.92 ± 0.45
KN101	1.00 ± 0.43	7.83 ± 0.37
KN102	1.08 ± 0.36	5.42 ± 0.40
KN103	0.75 ± 0.28	3.42 ± 0.67
KN105	1.75 ± 0.46	5.67 ± 0.48
KN107	0	2.17 ± 0.73
KN109	2.50 ± 0.56	7.75 ± 0.68
KN110	4.33 ± 0.93	6.75 ± 0.37
KN111	1.50 ± 0.45	6.83 ± 0.65
KN112	3.08 ± 0.95	5.33 ± 0.67
KN113	3.67 ± 0.84	7.42 ± 0.47
KN114	1.42 ± 0.48	7.25 ± 0.79
KN115	1.08 ± 0.31	6.83 ± 0.46
KN117	1.50 ± 0.47	5.75 ± 0.55
KN119	4.00 ± 0.81	4.17 ± 0.78
KN120	1.92 ± 0.50	7.17 ± 0.27
KN201	3.33 ± 0.83	4.83 ± 0.60
KN202	1.17 ± 0.44	3.67 ± 0.43
KN203	0.50 ± 0.23	2.25 ± 0.68
KN206	0.58 ± 0.40	5.67 ± 0.88
KN208	5.08 ± 0.48	5.67 ± 0.54
KN209	1.00 ± 0.37	4.17 ± 0.71
KN210	2.17 ± 0.47	6.58 ± 0.38
KN211	3.33 ± 0.86	3.67 ± 0.96
Mean	1.85 ± 0.25	5.35 ± 0.31

[a] Old and young males are 28 and 3 days old, respectively.

(49.6%). The result means that the genetic variability in mating activity at the old age is much higher than that at the young age, suggesting that virility at the old age is not subjected to selection as is young virility. Three lines (KN005, KN020, KN107) exhibited male sterility due to aging. Although young males in these lines are reproductively active, old males are completely sterile. It can be concluded that the male sterility due to aging is heritable. Males showing a high virility at the young age tend to become sterile when they have aged. One finding is that there is no significant correlation between the mating activity at the young age and that at the old age ($r = 0.265$). If three sterile lines at the old age are excluded from the calculation, the correlation coefficient becomes much smaller ($r = 0.082$). Spearman's rank correlation coefficient also proves

Table II. Analysis of Variance for Male Mating Activity in Homozygous Lines

Source	df	SS	MS	*F*
Age	1	2124.507	2124.507	520.62*
Strain	28	980.127	35.005	8.58*
Interaction	28	576.827	20.601	5.05*
Error	638	2603.500	4.081	
Total	695	6284.960		

* Significant at the 0.01 level.

not to be statistically significant ($r_S = 0.263$). When these sterile lines are excluded, the value decreases to almost zero ($r_S = 0.050$). These results imply that young males manifesting a high mating activity do not necessarily remain as sexually active after they have aged, and *vice versa*.

Two-way analysis of variance for male mating activity in Series A indicates that both age and strain differences are significant (Table II). The significant strain difference also suggests the genetic nature of this character. The significance of the interaction is expected, to some degree, since the correlation between old and young males is not statistically significant.

The frequency distribution of virility for all 348 males at the old and young ages under a homozygous condition is given in Fig. 1. Mating times at the old age differ significantly from a Poisson distribution. Males exhibiting a high virility are more frequent than expected from a Poisson distribution.

Figure 2 represents the frequency distribution of old and young males under a heterozygous condition. Some young heterozygous males were found to mate with as many as 12 females within 24 h (observation of two remaining females). The mean mating activity in old males was 3.76 $\pm$ 0.17, significantly lower than that (7.67 $\pm$ 0.21) in young males ($P < 0.001$). Genetic variability, measured by the coefficient of variation, was much higher in old males than in young males (65.1 vs. 28.2%). This result also suggests that virility at the old age may be less subject to selection. The tendency for higher genetic variability in old virility than in young virility was also seen in *D. virilis* (Aigaki and Ohba, 1984).

Heterozygous males are sexually more vigorous than homozygotes for old and young males. These differences between heterozygotes and homozygotes are statistically highly significant ($P < 0.001$).

Thus, the mating activity in 28-day-old (old) males is much lower than that in 3-day-old (young) males.

The relative mating activity ratio of homozygotes to heterozygotes for old males [0.49 (= 1.85/3.76)] is much lower than that (0.70) for young

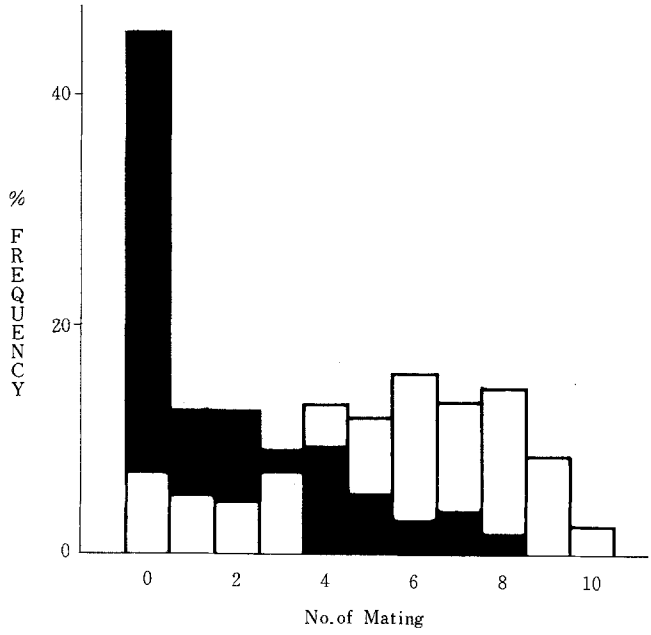

Fig. 1. Overlapped frequency distribution of mating activity in "old" and "young" homozygous males. The frequency of old and young males is given by the hatched and open bars, respectively.

males. The virility reduction in homozygous old males seems to be much greater than in young ones. It is quite probable that a great many deleterious genes, which are expressed only at an old age, are concealed in natural populations (Smith, 1978). These genes are almost free from natural selection, even under a homozygous condition, since they are expressed after males have passed through a substantial reproductive period. These genes do not constitute any genetic load, in spite of deleterious effects on virility at an old age. The fact that there is no correlation in mating activity between old and young males favors this conjecture. If the longevity of homozygotes is relatively shorter than that of heterozygotes (Pearl *et al.*, 1923; Clarke and Maynard-Smith, 1955), aging effects on male mating activity would be more severe in homozygotes than in heterozygotes. Brittnacher (1981) reported that the homozygote/heterozygote virility ratio for 2-day-old (young) males was 0.50 in *D. melanogaster* and 0.73 in *D. pseudoobscura*, respectively.

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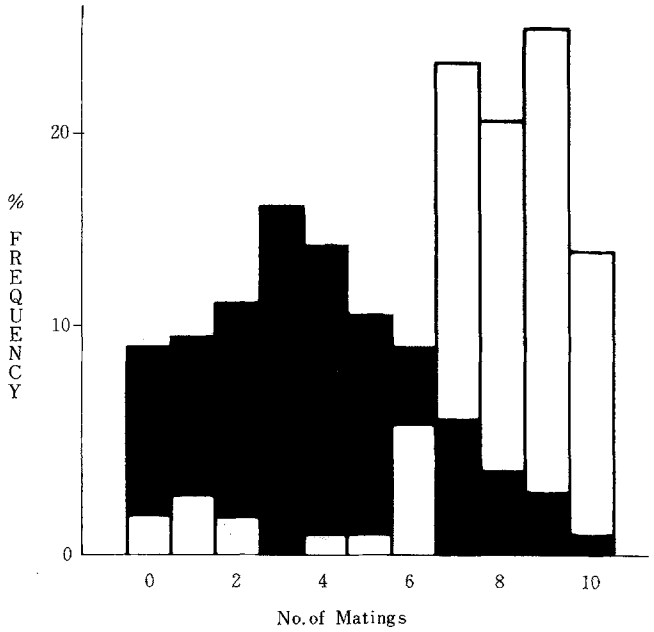

Fig. 2. Overlapped frequency distribution of mating activity in "old" and "young" heterozygous males. The frequency of old and young males is given by the hatched and open bars, respectively.

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