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The Effect of Inbreeding on Male Mating Ability in *Drosophila melanogaster*

W. W. PENDLEBURY and J. F. KIDWELL

Division of Biological and Medical Sciences, Brown University, Providence, R.I. (U.S.A.)

Summary. A set of experiments designed to measure the effect of rapid inbreeding on three components of male mating ability — competitive mating ability, mating rate and fertility — in *D. melanogaster* were conducted. A drastic reduction in competitive mating ability and mating rate was observed at a rather low level of inbreeding ($F = 0.25$), but the effect of further inbreeding was small. The results with respect to the effect of inbreeding on sperm quantity and quality were ambiguous due to an unsatisfactory experimental design.

The well known decline in reproductive fitness due to inbreeding of cross-fertilized species has been observed in numerous experiments with *Drosophila melanogaster*. Gowen (1952) found that inbred flies were inferior to outbreds in all aspects of female fecundity. Temin (1966) studied a sample of 237 nonlethal second chromosomes taken from a wild population in Madison, Wisconsin. When homozygous in males 8.4% were completely sterile and 24.1% were partially sterile; the corresponding figures for females are 4.2% and 30.7%, respectively. Her data indicated that a population made homozygous for all nonlethal second chromosomes would retain, on the average, about 90% normal fertility and one made homozygous for all nonlethal second chromosomes except those causing complete sterility would have about 95% fertility. Sved (1971) sampled second chromosomes from wild populations at five wineries located within a few miles of each other in New South Wales, Australia. Twenty-five population cages were established, each containing a different wild-type second chromosome and the marker chromosome Cy. He concluded that, overall, homozygosity of the entire second chromosome causes a depression in fitness relative to Cy/+ heterozygotes of the order of 85%. Sperlich and Karlik (1970) have done a similar experiment.

Parsons (1964) made a complete set of diallel crosses among six inbred lines that had been full-sib mated for more than 120 generations. Males and females of the same inbred line or cross were single pair mated and observed for 40 minutes. Crossbred pairs had a much higher mating frequency, especially during the first 10 minutes, than inbred pairs. Fulker (1966) conducted two experiments designed to study the effect of inbreeding on several aspects of male mating ability. In the first, inbred males of six lines and hybrid males from six of the 30 possible crosses among them were tested. Each male was exposed to six females, one of each inbred line, for 12 hours. Four traits were observed: 1) the time to first copulation, 2) the number of copulations, 3) the number of copulations resulting in fertilization and 4) the total number of progeny produced. Crossbred males were superior to inbreds for all four traits. Moreover, the traits were highly correlated. In the second experiment a 6×6 complete diallel cross was made and the males tested as before, except that only the number of copulations resulting in fertilization during the first 12 hours was scored. Crossbred males were again superior to inbreds.

Latter and Robertson (1962) used a competitive-index method to measure overall fitness (egg laying, larval survival and male mating ability) and observed a rapid decline in the index with continued full-sib mating. We are not aware of other experiments designed specifically to study the effect of inbreeding on male competitive mating ability, i.e. inbred males competing with outbreds. Robertson (1970) has noted that this may be of great importance in experiments on disruptive selection. The experiments discussed in this paper were designed to estimate the effect of rapid inbreeding on male competitive mating ability, mating rate and fertility.

Materials and Methods

In order to provide two populations that were homozygous for different alleles at each of two loci, but were otherwise similar and at or near genetic equilibrium, the recessive mutant ebony, *e* (3-70.7) was alternately backcrossed and crossed *inter se* into each of seven wild type laboratory stocks for three cycles. The 49 possible crosses were then made among the resulting seven homozygous ebony stocks and a large sample of males and virgin females of each used to establish a cage population. The second population was established in the same way, except that the mutant sparkling-poliert, *spa*^{pol} (4-3.0), referred to as polished, was used. These mutants were selected because they are readily observed; preliminary experiments indicated they have little or no affect on male mating ability in competition with each other; and the two mutant genes had recently been backcrossed into the same inbred line. As a result of this procedure

the origin of the 4th chromosome was different for the two populations and they probably differed in a short interval of chromosome 3 around the ebony locus. At the start of this work the cage populations had been maintained under a constant regime for nearly a year at an average size of about 3,000 individuals.

Within each marker population, a series of inbred lines were made with 1, 3, 6 and 14 consecutive generations of full-sib mating respectively. The coefficients of inbreeding (Wright, 1922), F, were .250, .500, .734 and .951. A different set of 24 lines was initiated for each level of inbreeding, and carried without reserves for the first three generations. At the two higher levels 10 replicates of the surviving lines were made in each generation, and one of the vigorous surviving replicates chosen at random.

The females used in all tests were heterozygous $e/+$; $spa^{pol}/+$ and were produced by crossing inbred lines with different markers. In all comparisons the marker genotype of each female's mate could be unambiguously determined by a progeny test. No double matings involving both markers were detected, as expected from the short exposure periods. The outbred males were produced from reciprocal crosses between two inbred lines carrying the same marker. The level of inbreeding of these lines varied from F = .672 to F = .940.

All measurements for a given level of inbreeding were made at one time, and each of the four levels of inbreeding measured at a different time. Hence effects due to level of inbreeding are confounded with any effects peculiar to the time of measurement. There is, however, no evidence or reason to suspect that the latter are other than negligible. A randomized complete block design would have been preferable, but was precluded by the added labor requirement.

A standard corn meal-molasses-agar medium seeded with live yeast was used. Cultures were made in either 1/2-pint milk bottles or 40cc shell vials, as appropriate, and were maintained at 25 $\pm$ 1 °C. All flies used in the tests were collected within eight hours of eclosion and stored either singly or in appropriate groups for five days prior to the test matings.

Male competitive mating ability was measured in two ways: either two males competing for a single female, or 20 males (10 of each marker genotype) competing for 10 females. Four of the six possible comparisons were made for both tests: 1) inbred polished vs. inbred ebony; 2) inbred polished vs. outbred ebony; 3) outbred polished vs. inbred ebony; 4) outbred polished vs. outbred ebony. Since matings were determined by classifying progeny, the two most useful direct comparisons, inbred vs. outbred polished and inbred vs. outbred ebony could not be made.

In the case of two competing males, thirty pairs of males and 30 single females were used in each of the four comparisons. The males were collected as a pair and stored in vials, and the females stored singly. The two males were transferred without etherization to the vial containing the female for two hours. The flies were then etherized, the males discarded and the female transferred to a fresh vial. The progeny of the fertile matings were classified to determine the males' genotype.

For the twenty males competing for ten females, three sets of ten females and ten males of each type were stored in vials. The flies of each set were transferred without etherization to a 1/2-pint milk bottle for two hours. They were then etherized, the males discarded, and the females transferred to individual vials to produce the test progeny.

Male mating rate was measured by exposing a single male to five females. The exposure period was 30 minutes for the first test (F = 0.734)and one hour for the others. Four types of male were compared: inbred or outbred; ebony or polished. Twelve replicates were made. Males were stored individually in vials and females in sets of five. Each male and his five females were transferred without etherization to a 1/2-pint milk bottle for the mating period. They were then etherized, the male discarded and the females transferred to individual vials to determine whether or not progeny were produced.

The male fertility test was designed to measure the number of adults produced by the sperm of a single copulation. A single male was transferred to a vial with a single female for thirty minutes for one level of inbreeding (F = 0.734) and one hour for the others. The female was then transferred, without etherization, to a fresh vial, and subsequently transferred to fresh vials at 48-hour intervals for 30 days. The total number of adults produced was counted. In all cases vials of the last two periods produced no adults. Fifteen males of each of the four types, inbred or outbred and ebony or polished were tested.

Results and Discussion

I. Male Competitive Mating Ability

The original data for both tests are presented in Table 1. Statistical treatment of the data was

Table 1. *The outcome of competition between males for females of genotype +/e, +/pol*

Level of Inbreeding	Successful Males											
	I pol vs. I e			I pol vs. O e			O pol vs. I e			O pol vs. O e		
	pol	e	no progeny	pol	e	no progeny	pol	e	no progeny	pol	e	no progeny
	Two males competing for a single female											
0.250	11	9	10	5	22	3	19	7	4	14	11	5
0.500	10	17	3	4	22	4	21	7	2	15	10	5
0.734	10	10	10	3	20	7	21	5	4	11	16	3
0.951	8	12	10	6	14	10	22	5	3	13	9	8
	Twenty males competing for ten females											
0.250	12	16	2	3	26	1	19	11	0	11	15	4
0.500	10	20	0	10	18	2	—	—	—*	14	12	4
0.734	13	13	4	8	20	2	17	11	2	10	17	3
0.951	11	8	11	2	20	8	18	9	3	14	15	1

I = Inbred; O = Outbred
* No data

accomplished by application of the appropriate chi square method.

a) *Two males competing for a single female:* Consider first the four comparisons of outbred polished males competing with outbred ebony males. The proportion of sterile females did not vary significantly among the four trials and is estimated as 0.175 ± 0.035. Polished males mated 44.2% of the females and ebony males mated 38.3%. The difference was not significant, but due to the restricted numbers the ability of the test to detect small but real differences is low. The pooled data for the four outbred comparisons can be added to the four inbred polished vs. inbred ebony comparisons as a zero level of inbreeding. These data suggest that the proportion of noninseminated females is lower for the outbreds than for the inbreds but that there is no difference among the four levels of inbreeding (0.175 ± 0.035 vs. 0.275 ± 0.041). To summarize, these data indicate: a) The two markers have no effect on mating ability when males of both genotypes are outbred or inbred to the same degree; b) one generation of full-sib mating results in a significant decline in a male's competitive mating ability, but no further decline results from increased inbreeding.

Observed differences in the number of females that were sterile or mated to either polished or ebony males did not differ significantly over the four levels of inbreeding for the inbred polished vs. outbred ebony comparisons, i.e. there was no significant interaction. On the basis of the preceding analysis we attribute the difference between the proportion of females mated to inbred polished and to outbred ebony males (0.15 ± 0.03 vs. 0.65 ± 0.04) to the effect of inbreeding and not to the two marker genotypes. The proportion of sterile females, 0.20 ± 0.04, is less than that observed when both males are inbred, but greater than when both males are outbred, as might be expected.

The same analysis was made for the outbred polished vs. inbred ebony matings. Again there was no significant interaction. The proportion of matings by outbred polished males was 0.69 ± 0.04 while that for inbred ebony males was 0.20 ± 0.04, in good agreement with the preceding results.

Since the proportion of matings by inbred (or outbred) males does not differ significantly between the two marker genotypes, the best estimate is obtained from the combined data. The proportion of females mated by inbred or outbred males or unmated are 0.175 ± 0.025, 0.671 ± 0.030 and 0.154 ± 0.023, respectively. The most interesting and important results of these comparisons are: 1) the large reduction in male competitive mating ability due to a moderate level of inbreeding, and 2) the failure of higher levels of inbreeding to have any further effect.

b) *Twenty males competing for ten females:* The proportion of sterile females or of those mated to outbred ebony or outbred polished males does not differ significantly over the four trials. The proportion of sterile females is 0.100 ± 0.027. Polished males mated 40.8 ± 4.5 percent of the females and ebony males mated 49.2 ± 4.6 percent. The difference is not significant, but again the ability of the test to detect small but real differences is low. The pooled data for the four outbred comparisons were added to the four inbred polished vs. inbred ebony comparisons as a zero level of inbreeding. The proportion of sterile females at the highest level of inbreeding is significantly greater than at the other levels. There were fewer matings by ebony males than expected in each of the three replicate mating bottles. There is no apparent explanation for this observation. The data are inadequate to justify the obvious speculation of a threshold effect at the higher level of inbreeding.

Except for the highest level of inbreeding, the difference in the proportion of sterile females between the inbreds and the outbreds is small and not significant. This is in marked contrast to the case of two males competing for a single female. Again omitting the highest level of inbreeding, the proportion of females mated by polished and by ebony males does not vary over the levels of inbreeding. The per cent of females mated by polished males, 40.0 ± 3.4, is significantly lower than the per cent mated by ebony males, 51.4 ± 3.4. This result also contrasts with that obtained for two males competing for a single female.

The proportions of sterile females, or those inseminated by inbred polished or by outbred ebony males differ significantly among the four levels of inbreeding, but there is no consistent pattern. At the $F = 0.500$ and $F = 0.734$ levels there are more inseminations by inbred polished matings than expected. Detailed examination of the data for the three replicate mating bottles suggests nothing unusual. The exceptional results at the $F = 0.951$ level were largely due to one mating bottle, from which there were no inseminations by polished males, three by ebony males and seven sterile females. There is, however, no obvious reason for this result. It is clear that the inbred ebony males inseminated fewer females than did the outbred polished males at all levels of inbreeding. The results are consistent with the previous observation that, over the range of $F = 0.250$ to $F = 0.951$, there is no relation between level of inbreeding and male competitive mating ability.

Part of the data for the $F = 0.500$ level in outbred polished versus inbred ebony comparisons was lost, and the rest is considered unreliable as the result of inadvertant faulty laboratory technique. The per cent of females inseminated by outbred polished or inbred ebony males or sterile did not differ significantly over the three levels of inbreeding and for the pooled data were 60.0 ± 5.2; 34.4 ± 5.0, and 5.6 ± 2.4, respectively.

Inbred ebony males consistently inseminated more females than did inbred polished males in the reciprocal test. The same general trend was noted in the case of two males competing for a single female. The suggestion that inbred ebony males are better competitors against outbred polished males than inbred polished males are against outbred ebony males cannot be dismissed.

The more important conclusions suggested by these data and analysis are:

1) There is a drastic reduction in male competitive mating ability with a rather low level ($F = 0.250$) of inbreeding. The effect of further inbreeding is slight. This result is contrary to that of Latter and Robertson (1962), who found an exponential decline in their male × female competitive index with increasing values of F.

There are at least three possible explanations for the difference: a) The degree of homozygosity in the inbreds is less than indicated by calculated F. At the two higher levels the procedure of making ten replicates for each line and chosing one of the fertile matings permitted a rather large amount of selection. b) The results can be attributed to sampling error and low test power due to small numbers. The consistency of the results argues against this interpretation. c) The results are accurate and there is a "threshold" effect. One might reason that full-sib matings are not uncommon in nature, but matings resulting in higher levels of inbreeding are rare. Hence mechanisms discriminating against this level of inbreeding would be highly developed.

2) The effect of the marker genes used is small. There is, however, evidence that ebony is a slightly better competitor than polished.

3) The proportion of noninseminated females is higher in the case of two males competing for a single female when both are inbred than in the others, among which there is little difference. This effect was not observed in the case of 20 males competing for 10 females.

II. Male Mating Rate

The number of females mated by each male was expressed as a percent of the total (5) to which he was exposed. The means of the twelve males for each marker genotype × inbreeding status subclass is given in Table 2 for each level of inbreeding. The difference between the 30-minute exposure ($F=0.734$) and the one-hour exposure was small and insignificant and is ignored. The datum for each male was transformed to angles, and the analysis of a 2 × 2 factorial experiment in a completely randomized design completed for each level of inbreeding.

Table 2. *The number of outbred females inseminated out of 60, when kept in groups of 5 for one hour with a single ♂*

	Inbred ♂		Outbred ♂	
F	*pol*	*e*	*pol*	*e*
0.250	7	7	15	19
0.500	7	7	14	13
0.734*	8	7	13	13
0.951	7	9	16	12

* 30 mins. only

There are no differences between marker genotypes when both are outbred or inbred to the same level. There is a large effect of inbreeding, but it is the same for all levels of inbreeding. This result is consistent with that observed for male competitive mating ability, and the same conclusions apply.

III. Male Fertility

The number of males (of 15 possible) that inseminated their mate and the mean number of progeny and standard error per inseminated female is presented in Table 3. The difference between the 30-minute exposure ($F = 0.734$) and the one-hour exposure was small and insignificant for both measures and is ignored.

The number of males that inseminated their mate may be considered a measure of mating rate rather than of fertility. The proportion of outbred polished and outbred ebony males that inseminated their mates did not differ significantly among the four experiments and were 0.767 ± 0.055 and 0.833 ± 0.048, respectively, for the pooled data. The difference between these two proportions is not significant.

The proportion of matings by polished and by ebony males did not differ over the four levels of inbreeding and were 0.500 ± 0.065 and 0.350 ± 0.062. These are significantly different from each other and from the corresponding outbred proportions.

The conclusions to be drawn from these data are: 1) Outbred males mate more rapidly than inbred

Table 3. *The number of outbred females inseminated when kept with a single male for one hour, out of 15 exposed, and the average progeny number of fertile matings*

	Inbred		Outbred	
F	*pol*	*e*	*pol*	*e*
0.250	8 34.0 ± 9.1	5 27.4 ± 12.2	10 101.6 ± 11.5	14 123.5 ± 20.9
0.500	4 74.5 ± 16.7	5 92.0 ± 12.8	13 113.2 ± 8.6	10 51.9 ± 8.7
0.734*	8 172.1 ± 47.3	8 129 ± 37.8	13 189.5 ± 34.1	14 107.6 ± 20.7
0.951	10 59.5 ± 19.1	3 38.3 ± 9.3	10 56.5 ± 6.5	12 59.7 ± 7.4

* 30 mins. only

males; 2) outbred polished males tend to mate a little more slowly than outbred ebony males; 3) there is a drastic decline in mating rate with a moderate amount of inbreeding, but no further decline at the higher levels of inbreeding investigated.

A conventional least squares analysis of variance of a 2×2 factorial experiment with unequal subclass numbers was applied to the fertility data at each level of inbreeding. At the $F = 0.250$ level outbred males produced more progeny than inbreds. At the $F = 0.500$ level there was a significant genotype $\times$ inbreeding interaction. Inspection of Table 3 indicates that the interaction is due to the relatively low production of females inseminated by inbred ebony males. There is no obvious reason for this. No other effects were significant.

This measure of male fertility is based on the assumption that females that are inseminated only once and then "brooded" or transferred to new vials would produce enough eggs to exhaust the supply of viable sperm. This assumption may not be valid. It has generally been observed that fecundity is stimulated by the presence of a male. The low number of progeny suggests that none of the females produced enough eggs to exhaust the supply of viable sperm. It has also been suggested that inbred males aged five days might develop enough sperm so that the number in a first ejaculate is equal to that of outbred males. Hence we are reluctant to interpret these observations as indicating that inbreeding does not affect the quantity or quality of sperm. Maynard Smith (1956) found the quantity and quality of sperm produced by inbred *D. subobscura* males inferior to that produced by outbred males. Better experiments are possible and should be done, e.g., sperm counts could be made, females might be accompanied by a sterile male, or males should be tested at 12 or 24 hours rather than 5 days.

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Mr. W. W. Pendlebury
Dr. J. F. Kidwell
Division of Biological and Medical Sciences
Brown University
Providence, R. I. 02912 (USA)