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Effect of rate of inbreeding on inbreeding depression in *Drosophila melanogaster*

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Summary. This experiment was designed to study the relationship between rate of inbreeding and observed inbreeding depression of larval viability, adult fecundity and cold shock mortality in *Drosophila melanogaster*. Rates of inbreeding used were full-sib mating and closed lines of $N = 4$ and $N = 20$. Eight generations of mating in the $N = 20$ lines, three generations in the $N = 4$ lines and one generation of full-sib mating were synchronised to simultaneously produce individuals with an expected level of inbreeding coefficient (F) of approximately 0.25. Inbreeding depression for the three traits was significant at $F = 0.25$. $N = 20$ lines showed significantly less inbreeding depression than full-sib mated lines for larval viability at approximately the same level of F. A similar trend was observed for fecundity. No effect of rate of inbreeding depression was found for cold shock mortality, but this trait was measured with less precision than the other two. Natural selection acting on loci influencing larval viability and fecundity during the process of inbreeding could explain these results. Selection is expected to be more effective with slow rates of inbreeding because there are more generations and greater opportunity for selection to act before $F = 0.25$ is reached. Selection intensities seem to have been different in the three traits measured. Selection was most intense for larval viability, less intense for fecundity and, perhaps, negligible at loci influencing cold shock mortality.

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Introduction

The random changes of gene frequencies which cause inbreeding depression are opposed by natural selection. Consequently, slower rates of inbreeding should be less effective in causing inbreeding depression than more rapid rates of inbreeding. This is because, at a slow rate of inbreeding, there are many generations during which selection can act before a given inbreeding coefficient is reached.

The magnitude of this effect will depend on the selection coefficients. If inbreeding depression is caused by very many loci, each with small selection coefficients, then selection will be ineffective in opposing inbreeding, and equal depression is expected for different rates of inbreeding at the same level of inbreeding coefficient.

Tantawy and Reeve (1956) found no consistent effect of rate of inbreeding on inbreeding depression in *Drosophila melanogaster*, whereas Tantawy (1957 a, b) did find that sib-mating caused more depression than double-first-cousin mating when the same level of inbreeding co-efficient (F) was reached. However, Tantawy (1957 a, b) found no inbreeding depression with any mating system until F reached 0.50 or 0.75.

This is relevant to livestock production. A major cause of heterosis when breeds are crossed is thought to be recovery of the inbreeding depression which has occured in each breed since they diverged (Falconer 1981). Goddard and Ahmed (1982) suggested that this inbreeding, and hence heterosis, can be predicted from gene frequencies at marker loci. However, the rate of inbreeding

within breeds of livestock is generally slow, so the heterosis in their crosses may be less than expected. Natural populations are also often large, so the inbreeding depression from which they suffer, and the heterosis observed in crosses between populations, may be less than would be predicted from the dispersion of gene frequencies for neutral alleles.

The experiment reported here was designed to study the relationship between rate of inbreeding and observed inbreeding depression in *Drosophila melanogaster* at a level of inbreeding (F = 0.25) that might be reached by breeds of livestock. Traits measured were adult fecundity, larval viability and cold shock mortality.

Materials and methods

Flies and mating systems

Flies from a cage population of about 5,000 flies established from 64 wild inseminated females trapped one year earlier at a site in Townsville were used for the experiment.

Rates of inbreeding used were full-sib mating and closed lines of $N = 4$ and $N = 20$. An inbred line C *spa* D, outcrosses and a closed line of $N = 100$ were used as controls. The C *spa* D line was obtained from R. Frankham's laboratory of the School of Biological Sciences, Macquarie University, Australia. It was derived from a Canberra population and was marked with the fourth chromosome recessive sparkling^{poliert}. Eight generations of mating in the $N = 20$ lines, three generations in the $N = 4$ lines and one generation of full-sib mating were synchronised to simultaneously produce individuals with an expected F of approximately 0.25.

Base flies of $N = 4$ and full-sib mated lines were allowed a probation period of 4 days after withdrawal from the cage to ensure that already inseminated females had rid themselves of all eggs fertilised by previous matings. All flies were observed to mate within 6 h of withdrawal from the cage and, based on the work of Gromko and Pyle (1978), after 4 days all eggs laid should be from the mating after withdrawal from the cage. No probation period was allowed for the $N = 20$ or $N = 100$ lines. Consequently, the base generation of each replicate of these lines was assumed to be 1½ times the actual number of flies withdrawn from the cage. The $N = 100$ line was established at the same time as the $N = 20$ lines but was maintained in five culture bottles, each containing 20 flies (ten of each sex), to ensure that equal selection pressure acted on them and the $N = 20$ lines throughout the period of experimentation. After each generation, 100 offspring of the $N = 100$ line (ten of each sex from each of the five culture bottles) were pooled and randomly reallocated to each of the five culture bottles for the next generation. In this way, randomness of mating was preserved. The number of lines for each mating system and the number of replicates measured for each trait are presented in Table 1. The outcrosses used as controls in the cold stress experiment were produced by mating unrelated flies from the families being reared as parents for the full-sib matings.

Calculation of inbreeding coefficient

It was assumed that the effective population size (Ne) was 70% of the actual number (N) in each closed population (Kerr and Wright 1954 a, b; Wright and Kerr 1954; Buri 1956).

Table 1. Mean performance of the mating systems

Trait	Mating system	Lines	Replicates per line	Mean performance ± SE	
Larval viability (%)	$N = 100$	1	10	85.6 ± 2.4	
	$N = 20$	20	2	77.6 ± 2.1	
	$N = 4$	20	2	73.3 ± 2.2	
	Full-sib	30	2	66.7 ± 3.6	
	Inbred (C *spa* D)	1	10	58.8 ± 2.7	
Fecundity (eggs/day)	$N = 100$	1	20	91.7 ± 2.5	
	$N = 20$	20	4	75.7 ± 2.6	
	$N = 4$	20	4	73.4 ± 2.2	
	Full-sib	30	4	69.8 ± 3.4	
	Inbred (C *spa* D)	1	20	45.6 ± 5.5	
				male	female
Cold shock mortality (% dead)	Outbred	15	4	74.3 ± 3.4	56.2 ± 4.8
	$N = 20$	4	4	87.3 ± 6.7	74.9 ± 9.5
	Full-sib	20	4	82.4 ± 3.7	76.2 ± 5.2

$N = 20$ and $N = 100$ lines. Inbreeding coefficient after eight generations was estimated from the formula given by Falconer (1981) as

$$F = 1 - (1 - \Delta F)^t \quad (1)$$

where $\Delta F = 1/2t\,[1/N_1 + 1/N_2 + \ldots + 1/N_t]$ approximately;

where

ΔF increase in F per generation,
t number of generations and
N_t effective number of flies in the t^{th} generation.
$N_1 = 1.5 \times 0.7\ N$
$N_j = 0.7\ N$ for $j > 1$.

From these equations, F equals 0.2432 and 0.0535 after eight generations for $N = 20$ and $N = 100$ closed lines, respectively.

$N = 4$ lines. For the early generations of a dioecious population, equ. (1) is not sufficiently accurate so the co-ancestry method of calculating the inbreeding co-efficient was used (Crow and Kimura 1970). This gives

$$F_t = F_{t-1} + (1 + F_{t-2} - 2F_{t-1})/2N_e$$

where F_t, F_{t-1} and F_{t-2} are the inbreeding coefficients at generations t, t−1 and t−2; and $F_k = 0$ for $K \leq 1$.

This gave an expected F value of 0.2934 (assuming an effective population size of 2.8) after three generations.

Full-sib mated lines. The expected F after one generation of full-sib mating is 0.25 (Falconer 1981).

Electrophoresis

An independent estimate of inbreeding coefficient in five of the 20 $N = 20$ lines was obtained from gene frequencies at electrophoretically-defined loci. The 10 loci used were Est-6, Acph, Adh, α-gpdh, Mdh, Odh, 6-pgd, Xdh and Pgm. The details of electrophoresis are described in Ehiobu (1985). The gene fre-

quencies were used to calculate Wright's F_{ST} by

$$F_{ST} = \frac{\sum_i \left[\sum_j p_{ij}^2 - 5\bar{p}_i\right]}{\sum_i 5\bar{p}_i(1-\bar{p}_i)}$$

where

p_{ij} frequency of gene i in population j
$\bar{p}_i$ mean frequency for gene i in the 5 populations
$\sum$ implies summation across alleles and loci.

An estimate of the amount of inbreeding since the lines diverged (F) can be derived from Nei (1976) as

$$F = \frac{F_{ST}}{1 + 1/n\, F_{ST}}$$

where

n = number of populations.

The standard error of F was calculated from the standard deviation of F_{ST} across loci.

Experimentation

Larval viability study. Larval viability was defined as survival from larva to adult stages. Each replicate consisted of 25 first-instar larvae (less than 12 h old), which were counted into vials with five to six ml well-yeasted media and allowed to stand undisturbed until the adults emerged. Emerged flies were counted and expressed as a proportion of the 25 larvae introduced into the vials.

Fecundity study. Flies emerging from the larval viability study were used for the fecundity study because these flies had experienced a standardized larval environment of low population density and, consequently, were fairly uniform in size. Males and virgin females were mated in single pairs for 48 h (in the analysis each mated pair formed a replicate). Subsequently, each pair was shaken into a new vial with blue feed every 24 h for 4 consecutive days. Eggs were counted using a low magnification (× 15) binocular microscope and average daily egg production was recorded for each pair of flies.

Cold shock mortality. Four independent replicates of 20 flies (two replicates for each sex) per line were matured for 2–3 days. Flies were shaken into clean vials and exposed to a temperature of 1 °C for 48 h. Percent mortality was recorded at the end of the treatment after allowing a 3 h recovery time at 25 °C.

Data analyis

Analyses of variance were carried out by least squares fitting the model

$$Y_{ijkl} = \mu + M_i + L_{ij} + e_{ijkl}$$

where

Y_{ijkl} = individual replicate fecundity, viability or cold shock mortality. (Viability and mortality replicate frequencies were angularly transformed to stabilize variance before analysis of variance, since these traits were binomially distributed.)
μ population mean
M_i effect of the i^{th} mating system ($i = 1, \ldots, 5$)
L_{ij} effect of the j^{th} line within the i^{th} mating system
e_{ijkl} residual random error term, assumed NID $(0, \sigma^2)$

For fecundity, a term $T_k (K = 1, 2)$ was added to the model to represent the effect of the time of day when flies were transferred to new vials. For cold shock mortality, a term $S_k (K = 1,2)$ was included in the model for the effect of sex.

The mating systems (full-sib, $N = 4$ and $N = 20$) compared in the analyses of variance did not reach exactly equal levels of inbreeding. To allow for this, the inbreeding depression per percent inbreeding was calculated for each mating systems by the formula

$$\text{depression per \% F} = D_i = \frac{Y_i - Y_c}{F_i - F_c}$$

where

$Y_i - Y_c$ mean performance of mating system i minus mean performance of controls
$F_i - F_c$ inbreeding coefficient of mating system i minus inbreeding coefficient of controls.

The standard error of D_i is

$$(V_i + V_c)^{0.5}/(F_i - F_c)$$

where

V_i sampling variance of Y_i; calculated as the mean square for lines within mating systems divided by the number of observations.

D_i estimates for different mating systems are not independent because they all involve the control performance y_c. This was taken into account in calculating the standard error of the difference between D_i and D_j by the formula

$$\left[\frac{V_i}{(F_i - F_c)^2} + \frac{V_j}{(F_j - F_c)^2} + \left[\frac{1}{F_i - F_c} - \frac{1}{F_j - F_c}\right]^2 V_c\right]^{0.5}$$

Results and discussion

Inbreeding depression

Mean fecundity, larval viability and cold shock mortalities of flies produced by the different mating systems at approximately F = 0.25 and for controls are presented in Table 1. Analyses of variance (Table 2) indicated a significant effect of mating system on mean larval viability, adult fecundity and cold shock mortality. Comparison of the inbred lines and the controls shows that inbreeding depression occurred for all the traits. This agrees with the findings of Latter and Robertson (1962) who reported a decrease of more than 50% in "competitive index" for *D. melanogaster* over an outbred control after one generation of full-sib mating. On the other hand, Tantawy (1952, 1957 a, b) found no inbreeding depression for body size and percent emergence until F was greater than 0.5. Tantawy used a population which had been maintained with 20 pairs for many generations and may have been purged of many deleterious recessives. Lines within mating systems varied significantly ($P < 0.01$) in mean inbreeding depression for the three traits. Female flies survived cold shock better ($P < 0.001$) than the males (Table 2).

Table 2. Analyses of variance[a]

Effects	Larval viability		Fecundity		Cold shock mortality	
	MS[b]	F	MS[b]	F	MS[b]	F
Mating system	0.210 (4)	3.67**	5 825 (4)	7.39**	0.925 (2)	4.38*
Lines within mating sytem	0.0573 (63)	3.54**	788 (63)	1.84**	0.211 (34)	5.59**
Time	–	–	830 (1)	1.95	–	–
Sex	–	–	–	–	0.696 (1)	18.44**
Error	0.0162 (84)		425 (227)		0.0377 (110)	

[a] Frequencies of viability and mortality were angularly transformed to radians for analysis
[b] Degrees of freedom in parentheses
* $P<0.05$
** $P<0.01$

Table 3. Effect of rate of inbreeding on inbreeding depression for larval viability and adult fecundity

Mating system	Expected inbreeding coefficient (%)	Increase in inbreeding coefficient from control (%)	Depression for larval viability per % F ± SE (%)[1]	Depression for adult fecundity per % F ± SE eggs/day)[2]
$N=100$ (control)	5.4	– –	–	
$N=20$	24.3	19.0	0.42 ± 0.17^{a}	0.84 ± 0.19
$N=4$	29.3	24.0	0.55 ± 0.14	0.76 ± 0.14^{b}
Full-sib	25.0	19.7	0.96 ± 0.22^{a}	1.11 ± 0.21^{b}

[1] Values which share the superscript [a] are significantly different ($P<0.05$)
[2] Values which share the superscript [b] are significantly different ($P<0.1$)

Effect of rate of inbreeding on inbreeding depression

Table 3 presents the depression of larval viability and adult fecundity per % F for the different rates of inbreeding. The $N = 20$ lines exhibited a significantly ($P < 0.05$) smaller depression than full-sib mated lines for larval viability. For fecundity, the trend was in the same direction and the difference between the $N = 4$ and full-sib lines approached significance ($P < 0.1$). Rate of inbreeding appeared to have no effect on inbreeding depression for cold shock mortality. The inbreeding depression per % F was 0.56% ± 0.23% for full-sibs and 0.65% ± 0.30% for $N = 20$ lines. However, depression of cold shock mortality of the $N = 20$ closed lines was not estimated as accurately as adult fecundity or larval viability, because only four of the lines were available for testing at the time of the experiment.

There are two possible explanations for the smaller depression of larval viability with the $N = 20$ than with full-sib mated lines. First, the random changes in gene frequency and increase in homozygosity caused by inbreeding will be opposed by natural selection. Milder rates of inbreeding allow more generations of selection to operate before the same expected level of F is reached.

Secondly, the assumption that $N_e = 0.7\,N$ for the $N = 4$ and $N = 20$ lines may be incorrect. If N_e was, in fact, greater than $0.7\,N$, then we would have overestimated F and underestimated the inbreeding depression per % F for these lines. The inbreeding coefficient calculated from gene frequencies at electrophoretically-defined loci was 0.278 ± 0.062 for the $N = 20$ lines. This is in good agreement with the expected value of F = 0.243 and so argues against this second explanation.

The effectiveness of selection in opposing inbreeding depression depends on both the rate of inbreeding and the selection coefficients operating at the loci under consideration. The higher the selection coefficients, the more effective selection will be. In this study we found that rate of inbreeding had the greatest effect on larval viability, a lesser effect on fecundity and no significant effect on cold shock mortality. This would be the expected result if the selection coefficients at loci controlling larval viability were the highest, and if little selection operated at loci controlling cold shock mortality. This is a plausible hypothesis. There are a number or lethal genes which affect larval viability. Also, natural selection for viability and fecundity cannot be prevented but, during the experiment, the flies were maintained at 25 °C, which should minimize selection for survival of cold shock.

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References

Buri P (1956) Gene frequency in small polpulations of mutant Drosophila. Evolution 10:367–402

Crow JF, Kimura M (1970) An introduction to population genetics theory. Harper and Row, New York

Ehiobu NG (1985) The prediction of heterosis. Unpublished Ph. D. thesis, Graduate School of Tropical Veterinary Science, James Cook University of North Queensland, Townsville, Australia

Falconer DS (1981) Introduction to quantitative genetics, 2nd edn. Longman, London New York.

Goddard ME, Ahmed AM (1982) The use of genetic distance between cattle breeds to predict the heterosis in crosses. Proc. 2nd World Congr on Genet Appl to Livestock Prod, Madrid 8: 377–382

Gromko MH, Pyle DW (1978) Sperm competition, male fitness and repeated mating by female *Drosophila melanogaster*. Evolution 32:588–593

Kerr WE, Wright S (1954 a) Experimental studies of the distribution of gene frequencies in very small populations of *Drosophila melanogaster*. I. Forked. Evolution 8:172–177

Kerr WE, Wright S (1954 b) Experimental studies of the distribution of gene frequencies in very small populations of *Drosophila melanogaster*. III. Aristapedia and spineless. Evolution 8:293–302

Latter BDH, Robertson A (1962) The effects of inbreeding and artificial selection on reproductive fitness. Genet Res 3:110–138

Nei M (1976) Mathematical models of speciation and genetic distance. In: Karlin S, Nevo E (eds) Population Genetics and Ecology. Academic Press, New York, pp 723–765

Tantawy AO (1952) Changes in genetic variability with different intensities of inbreeding. Drosophila Inform Serv 26:123–125

Tantawy AO (1957 a) Genetic variance of random-inbred lines of *Drosophila melanogaster* in relation to coefficients of inbreeding. Genetics 42:121–136

Tantawy AO (1957 b) Heterosis and genetic variance in hybrids between inbred lines of *Drosophila melanogaster* in relation to the level of homozygosity. Genetics 42:535–543

Tantawy AO, Reeve ECR (1956) Studies in quantitative inheritance. IX. The effect of inbreeding at different rates in *Drosophila melanogaster*. Z Indukt Abstam Vererbungsl 87: 645–667

Wright S, Kerr WE (1954) Experimental studies of the distribution of gene frequencies in very small populations of *Drosophila melanogaster*. II. Bar. Evolution 8:225–240