Effects on heterozygosity and reproductive fitness of inbreeding
with and without selection on fitness in *Drosophila melanogaster*

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Abstract. The effects of inbreeding, with (IS) and without selection (IO) for reproductive fitness, on inbreeding depression and heterozygosity were evaluated in 20 lines of each treatment inbred over seven generations using full-sib mating. The survival of lines was significantly greater in IS (20/20) than in IO (15/20). The competitive index measure of reproductive fitness was significantly lower in the inbred lines than in the outbred base population, but not significantly different in surviving IS and IO lines. There was a trend for higher fitness in the IS treatment as relative fitnesses were 19% higher in IS than IO for surviving lines and 59% higher for all lines. Heterozygosities were lower in the inbred lines than in the base population, and significantly higher in the IS than the IO lines. Consequently, the reduction of inbreeding depression in IS has been achieved, at least in part, by slowing the rate of fixation.

Key words: Reproductive fitness – Inbreeding depression – Heterozygosity – *Drosophila melanogaster* – Selection – Extinction

Introduction

Inbreeding, the mating together of individuals related by descent, leads to decreased heterozygosity, to divergence among lines, and to inbreeding depression in lines derived from outbred populations (see Falconer 1989). The deleterious effects of inbreeding are of major concern in developing inbred lines and in the maintenance of endangered species, especially those founded from, and maintained with, small population sizes (see Templeton and Read 1983).

An important question is: can selection on reproductive performance within inbred lines be used to alleviate inbreeding depression? Evidence on this point is conflicting. Richey and Mayer (1925), Shultz (1953), Bell et al. (1955), Abplanalp (1974) and Templeton and Read (1983), reported that selection was effective in reducing inbreeding depression in reproductive characters in maize, poultry, *Drosophila* and Speke’s gazelle, while Cornelius and Dudley (1974), Good and Hallauer (1977), MacNeil et al. (1984), Ehiobu et al. (1989) and Falconer (1989), made similar inferences for maize, Japanese quail, *Drosophila* and mice, based on less direct evidence. Conversely, Dickerson et al. (1954) and Bowman and Falconer (1960) reported no beneficial effect of selection on fitness during inbreeding in pigs and mice.

Selection on reproductive fitness traits has a low predicted response since heritabilities for these traits are low (see Gustafsson 1986; Mousseau and Roff 1987; Roff and Mousseau 1987; Falconer 1989). However, selection in inbred lines should have greater effectiveness than indicated by the heritabilities for fitness traits. Inbreeding increases homozygosity and so exposes deleterious recessives to more effective selection. Lopez-Fanjul and Villaverde (1989) showed that selection response for a fitness trait was 6.5 times greater in lines subjected to one generation of full-sib mating than in an outbred population and the realized heritability was 4.0 times greater. Further, selection within inbred lines will operate predominantly on large blocks.

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of genes in linkage disequilibrium, rather than on individual loci.

If selection on reproductive fitness in inbred lines is effective, it would be expected to act by slowing the rate of decline in heterozygosity. Theoretical work by Hayman and Mather (1953), Reeve (1955), and Reeve and Gower (1958) has shown that selection favouring heterozygotes directly or through associative overdominance can retard the rate of fixation under inbreeding. Rumball (1974) showed that the decline in allozyme heterozygosity in *Drosophila melanogaster* lines inbred by full-sib or double first-cousin mating was significantly slower than predicted by inbreeding theory, and that natural selection opposing fixation was responsible. Mina et al. (1991) found similar effects in poultry.

The aims of the present work were to evaluate the effects on heterozygosity and reproductive fitness, of inbreeding with and without selection on reproductive fitness, in replicate inbred lines. There was less inbreeding depression in the lines inbred with selection (less extinction of lines) and heterozygosities were higher in them.

**Materials and methods**

**Base population**

The outbred Armidale strain of *D. melanogaster* was founded from 402 wild inseminated females caught at Armidale, NSW, Australia, in February 1986 and maintained in a population cage with a population size of 3–4,000. A sample of 225 inseminated females was obtained from J. S. F. Barker in September 1986 and maintained using 20 bottles (approximately 25 pairs of parents/bottle) per generation on PS medium (Frankham et al. 1988). The experiment reported here commenced 2 years later.

**Inbred lines**

Two treatments were used, namely:

**IO, full-sib inbreeding without selection on reproductive fitness.** Twenty inbred lines were maintained.

**IO, full-sib inbreeding with selection for fitness.** Twenty of these inbred lines were maintained. The selection was based on competition of a premated female (full-sib mated) of the inbred line with four premated (to compound strain males) compound chromosome strain females [{C(2L), b; C(2R), cn bw}]. Five females were tested per inbred line in each generation and the most fit female selected on the basis of the highest ratio of wild-type to compound progeny. This test is related to the competitive index measure of reproductive fitness, except that it excludes the mating component of fitness.

Inbreeding was carried out for seven generations with the lines being maintained on PS medium at 25 °C.

**Reproductive fitness determinations**

Reproductive fitnesses in the IO and IS inbred lines (at generation 7) and in the Armidale base population were determined using the competitive index, which involved competing them with a marked compound chromosome strain [{C(2L), b; C(2R), cn bw}] as detailed by Frankham et al. (1988). As half of the inbred lines in each treatment were maintained on a cycle 1 week later than the other half, the fitness tests were done in two halves, 1 week apart, with base population controls being included in each week. To obtain sufficient inbred line flies for the fitness tests, five inbred cultures were set up from each inbred line and equal numbers of virgins (as far as possible) from each of the vials used in the fitness test. Two replicate bottles were set up for each inbred line test, each with 20 pairs of virgin inbred flies and 20 pairs of virgin compound strain flies. The parents were transferred to fresh bottles after 3 days and the total number of wild-type and compound flies from the original and transfer bottles pooled. For the base population controls, the flies used for each replicate came from a separate set of five pairs of randomly chosen parents. Twenty and 19 such groups were set up contemporaneously with the inbred lines at the two times.

**Electrophoresis**

Electrophoresis was used to examine the Armidale base population for variation at 16 loci (see Briscoe et al. 1992) known to be polymorphic in *D. melanogaster* populations (see O'Brien and MacIntyre 1978). Only alcohol dehydrogenase (Adh – EC 1.1.1.1) and α-glycerophosphate dehydrogenase (α-Gpdh – EC 1.1.1.8) showed variation (Briscoe et al. 1992). Five individuals from each IS and IO inbred line and 400 base population flies were typed for Adh and α-Gpdh using Cellogel (Chemtrun) electrophoresis. Runs used a Tris-EDTA-Borate-MgCl2 buffer (pH 7.8) and staining followed Richardson et al. (1986).

**Statistical analysis**

Fisher’s exact test was employed to test for differences in the numbers of surviving and non-surviving lines in the IS and IO treatments. Mann-Whitney non-parametric tests were used to compare competitive indices in inbreds with controls, IS with IO inbred lines, and to assess time effects (i.e., testing for differences between assessments done a week apart), since variances among inbred lines are expected to be greater than those among controls, the values of the ratio spanned the range from 0 to infinity (and so were non-normal), and ratios have difficult statistical properties. Mann-Whitney tests were done on competitive indices using the Minitab statistical package (Ryan et al. 1985). For the inbred lines the competitive index was computed from the pooling of flies from the two replicates per line. One control competitive index of infinity was entered as 9999 (and top rank 1) for analyses. The more powerful analysis of variance was performed on the reproductive fitness data for surviving IS and IO inbred lines. This was performed on the proportion of wild-type flies subject to the arcsin square root transformation as recommended by Jungen and Hartl (1979). A regression analysis of variance with indicator variables was used due to the unbalanced design (unequal numbers of surviving lines in the IS and IO treatments and missing replicates for two inbred lines). IS versus IO selection treatment effects were treated as random effects, time as a fixed effect, replicate inbred lines were nested within selection treatment and time, and duplicates nested within inbred lines.

Chi-squared tests were used to test for differences in gene frequencies and heterozygosities among IS, IO, and controls, and to test deviation from Hardy-Weinberg equilibrium genotype frequencies. All tests for differences between IS and IO and between controls and inbred lines were done as one-tail tests since the expectations are directional.