Theor Appl Genet (1993) 86:1023–1027

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

R. Frankham, G. J. Smith*, D. A. Briscoe

School of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

Received: 5 May 1991 / Accepted: 28 January 1993

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

Communicated by J. S. F. Barker

* *Present address:* Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia
Correspondence to: R. Frankham

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 heterozygosities 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 $2\frac{1}{2}$ 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 (Chemetron) electrophoresis. Runs used a Tris-EDTA-Borate-$MgCl_2$ 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.

Results

Loss of lines during the seven generations of inbreeding was significantly greater in the IO treatment (5/20) than in IS (0/20) ($P = 0.024$ using a one-tailed Fisher's exact test). The competitive index measures of reproductive fitness for both inbred and control groups are shown in Fig. 1. Controls had a substantially higher competitive index (Table 1) than the IS and IO inbred lines combined, the differences being significant (Table 2). Fitnesses of surviving lines in the IS and IO treatments were not significantly different with either the Mann-Whitney test (Table 2) or an F test (Table 3). Even though the difference in fitness between the surviving IS and IO inbred lines was not significant, the mean competitive index was 19% greater in the IS treatment than in the IO treatment, based on the total number of wild-type and compound flies emerging in all lines. When non-surviving IO lines were included, IS exceeded IO by 59%. Inbred lines within treatments showed large and significant differences (Table 3) such that the error term for the comparison was very large. Variances in surviving IO and IS lines for arcsin transformed fitness data did not not differ ($F_{14,19} = 1.03$, $P = 0.47$). Time effects were non-significant in all tests.

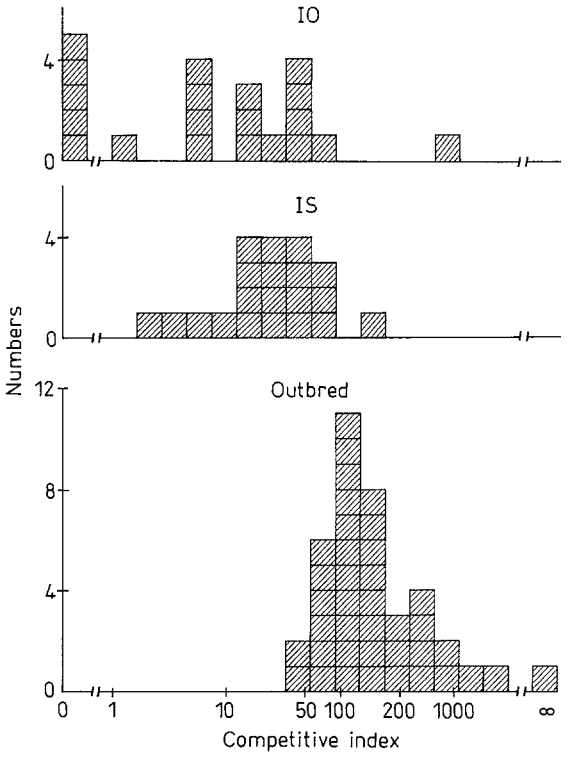

Fig. 1. Distributions of competitive index measures of reproductive fitness in the IO, IS, and outbred control treatments

Table 1. Mean reproductive fitness values for outbred control, IS (inbred selected), and IO (inbred non selected lines)

Line	Relative fitness[a]	CI[b]	Arcsin $\sqrt{p}$[c]
IS	0.124	17.33	1.346 ± 0.044[d]
IO (all)	0.078	10.88	0.997
IO (surviving)	0.103	14.51	1.329 ± 0.051
Outbred	1.000	140.20	1.493 ± 0.005

[a] Ratio of competitive index in the treatment to that in the outbred base population control
[b] CI = competitive index determined from the ratio of line flies to compound chromosome flies amongst emergences pooled over all replicates
[c] Arcsin square root (in radians) of the proportion of line flies emerging in competition with the compound chromosome strain
[d] Standard errors

Table 2. Analyses of reproductive fitness data using the Mann-Whitney test

Comparisons	W
Inbreds v controls	2065**
IS v IO	376
Time 1 v 2	1480

** $P < 0.01$ with a one-tailed test

Table 3. Analysis of variance of fitness measurements for inbred lines (deceased lines excluded)

Source	*df*	Mean square $\times 10^2$
IS v IO	1	0.446
Time	1	0.807
(IS v IO) $\times$ time	1	0.041
Replicate inbreds/ selection and time	31	4.135**
Duplicates within inbreds	33	0.503
Total	67	

**$P < 0.01$

Gene frequencies did not differ significantly among IS, IO and the Armidale control at either the α-Gpdh locus (contingency chi-square = 3.50, $P = 0.17$, $df = 2$) or the Adh locus (contingency chi-square = 3.82, $P = 0.15$). Genotype frequencies (Table 4) at the α-Gpdh locus differed significantly from 2pq Hardy-Weinberg equilibrium expectations in the IS (chi-square = 53.0) and IO (chi-square = 66.7) inbred treatments, but not in the control (chi-square = 1.8). Corrections were not made for small sample sizes in the inbred lines when testing for deviations from Hardy-Weinberg equilibrium (Cannings and Edwards 1969). That would only increase the size of the already significant deviations.

Heterozygosities at the α-Gpdh locus were significantly lower in the inbred lines than in the base population control. A similar, but non-significant, effect was

Table 4. Genotype frequencies for the α-Gpdh and Adh loci in the base population control and in the IS and IO inbred lines

Locus	Controls	IS	IO
α-Gpdh	H-W	H-W**	H-W**
FF	0.706	0.765	0.795
FS	0.279**	0.062	0.014
SS	0.015	0.173	0.192
n[a]	398	81	73
Adh			
FF	0.953	0.967	1.000
FS	0.048	0.033	0.000
SS	0.000	0.000	0.000
n	400	90	75

[a] Number of individual typed
H-W** $P < 0.01$ for test of deviation from Hardy-Weinberg equilibrium genotype frequencies for the α-Gpdh locus
** $p < 0.01$ for comparisons of heterozygosities between controls and combined inbreds

evident for the Adh locus where the extreme gene frequency meant that the test had little power. There was a non-significant trend towards higher heterozygosities in the IS treatment than in the IO treatment at both the α-Gpdh ($P = 0.16$ with Fisher's exact test) and Adh ($P = 0.13$) loci. However, when the results for these two loci were combined, the joint probability was 0.021 and significant.

Discussion

Reproductive fitness clearly declined with inbreeding. Such inbreeding depression has been extensively documented and discussed for a wide range of species (see Wright 1977; Charlesworth and Charlesworth 1987; Falconer 1989). Inbreeding depression in the competitive index after seven generations of full-sib inbreeding in the IO treatment was 92% for all lines and 90% for surviving lines, similar to the values reported by Latter and Robertson (1962) after the same amount of full-sib inbreeding (91% for all lines, and 84% for surviving lines).

Inbreeding depression was 88% for the IS inbred lines (all survived), so selection on fitness did not prevent a substantial inbreeding depression. However, selection on reproductive fitness during inbreeding significantly reduced this depression, by significantly enhancing the survival of lines (100% for IS and 75% for IO). Even though the difference in fitness between the surviving IS and IO inbred lines was not significant, the IS treatment had a competitive index 19% greater than that in the IO treatment, and a 59% advantage when all lines were included. It must be stressed that the effects of selection are underestimated as there is also selection in the IO lines, due to the use of replacements to maintain lines where the allocated parents did not reproduce, and due to natural selection within lines favouring heterozygosity (Rumball 1974; Mina et al. 1991). The intensity of selection in IS was greater in terms of the choice of parents to reproduce the lines and greater within these lines due to increased competition as a result of adding compound chromosome flies to the cultures.

These results demonstrate that selection on reproductive fitness in inbred lines reduces inbreeding depression in fitness. Other studies on this point have yielded conflicting results (see Introduction). However, the majority of studies have concluded that selection was effective, the exceptions being the studies by Dickerson et al. (1954) in pigs and by Bowman and Falconer (1960) in mice. Differences among studies could reflect the size of studies, the inbreeding levels achieved, the intensity of selection on fitness, different environmental variances, genotype × environmental interactions, different heritabilities of fitness, the base populations used, or combinations of these factors. The study by Dickerson et al. (1954) has several features that make its conclusions suspect. Firstly, selection with and without inbreeding was not compared, the effectiveness of selection having to be inferred from statistical partitioning. Secondly, selection was based on an index of several fitness and non-fitness characters. Thirdly selection intensities on any character were relatively weak. Fourthly, selection on litter size in mice and pigs is fraught with difficulties due to the negative environmental correlations between litter sizes of mothers and daughters (see Falconer 1965). Fifthly, line crosses were used as environmental controls and these may differ from inbred lines in their responses to environmental changes. While Bowman and Falconer (1960) failed to find significant effects of selection in inbred lines of mice on the rate of inbreeding depression, they attributed this to the weak selection they applied. Falconer (1989) subsequently concluded that such selection in inbred lines of mice was effective. Consequently, the majority of evidence indicates at selection on fitness during inbreeding can reduce inbreeding depression. This selection should be more effective with slower rates of inbreeding as used by Templeton and Read (1983) and Lopez-Fanjul and Villaverde (1989).

The lines inbred with selection on fitness showed a higher level of heterozygosity than lines inbred without the culling on fitness. Thus it appears that the alleviation of inbreeding depression due to selection on reproductive fitness has been achieved, at least in part, by slowing the rate of fixation. These observation have important implications in plant and animal breeding, in the production of inbred lines for experimental purposes, and in conservation biology.

Acknowledgements. We thank M. F. Graham and R. Davis for technical Assistance, A. Eyland for statistical advice and R. Frankham Jr. and two anonymous reviewers for comments on the manuscript. Revision of this manuscript was done while R. F. was at Arizona State University with P. W. Hedrick. This work was supported by an Australian Research Council grant.

References

Abplanalp, HA (1974) Inbreeding as a tool in poultry improvement. Proc 1st World Congr Genet Appl Livestock Prod (Madrid) 897–908

Bell AE, Moore CM, Warren DC (1955) The evaluation of new methods for the improvement of quantitative characteristics. Cold Spring Harbor Symp Quant Biol 20:197–211

Bowman JC, Falconer DS (1960) Inbreeding depression and heterosis of litter size in mice. Genet Res 1:262–274

Briscoe DA, Malpica JM, Robertson A, Smith GS, Frankham R, Banks R, Barker JSF (1992) Loss of genetic variation in large laboratory populations of *Drosophila melanogaster*: implications for the genetic management of captive populations. Conserv Biol 6:416–425

Cannings C, Edwards AWF (1969) Expected genotypic frequencies in a small sample: deviation from Hardy-Weinberg equilibrium. Am J Hum Genet 21:245–247

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268

Cornelius PL, Dudley JW (1974) Effects of inbreeding by selfing and full-sib mating in a maize population. Crop Sci 14:815–819

Dickerson GE, Blunn CT, Chapman AB, Kottman RM, Krider JL, Warwick EJ, Whatley JA, Baker ML, Lush JL, Winter LM (1954) Evaluation of selection in developing inbred lines of swine. Univ Missouri College Agriculture Res Bull 551, Columbia, Mo., USA

Ehiobu NG, Goddard ME, Taylor JF (1989) Effect of rate of inbreeding on inbreeding depression in *Drosophila melanogaster*. Theor Appl Genet 77:123–127

Falconer DS (1965) Maternal effects and selection response. In: Geerts SJ (ed) Genetics today, Proc XI Int Congr Genet, vol 3. Pergamon, Oxford, pp 767–773

Falconer DS (1989) Introduction to quantitative genetics, 3rd edn. Longman, Harlow, England

Frankham R, Yoo BH, Sheldon BL (1988) Reproductive fitness and artificial selection in animal breeding: culling on fitness prevents a decline in reproductive fitness in lines of *Drosophila melanogaster* selected for increased inebriation time. Theor Appl Genet 76:909–914

Good RL, Hallauer AR (1977) Inbreeding depression in maize by selfing and full-sibbing. Crop Sci 17:935–940

Gustafsson L (1986) Lifetime reproductive success and heritability: empirical support for Fisher's fundamental theorem. Am Nat 128:761–764

Hayman BI, Mather K (1953) The progress of inbreeding when homozygotes are at a disadvantage. Heredity 7:165–183

Jungen H, Hartl DL (1976) Average fitness of populations of *D. melanogaster* as estimated using compound-autosome strains. Evolution 33:359–370

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

Lopez-Fanjul C, Villaverde A (1989) Inbreeding increases genetic variance for viability in *Drosophila melanogaster*. Evol 43:1800–1804

MacNeil MD, Kress DD, Flower AE, Webb RP, Blackwell RL (1984) Effects of mating system in Japanese quail. I. Inbreeding and fitness. Theor Appl Genet 67:403–406

Mina NS, Sheldon BL, Yoo BH, Frankham R (1991) Heterozygosity at protein loci in inbred and outbred lines of chickens. Poultry Sci 70:1864–1872

Mousseau TA, Roff DA (1987) Natural selection and the heritability of fitness components. Heredity 59:181–197

O'Brien SJ, MacIntyre RJ (1978) Genetics and biochemistry of enzymes and specific proteins of *Drosophila*. In: Ashburner M, Wright TRF (eds) The genetics and biology of *Drosophila*, vol 2a. Academic Press, London, pp 395–551

Reeve ECR (1955) Inbreeding with homozygotes at a disadvantage. Ann Hum Genet 19:332–346

Reeve ECR, Gower JC (1958) Inbreeding with selection and linkage. II. Sib mating. Ann Hum Genet 23:36–49

Richardson BJ, Baverstock PR, Adams M (1986) Allozyme electrophoresis, a handbook for animal systematics and population studies. Academic Press, Sydney

Richey FD, Mayer LM (1925) The production of successive generations of self-fertilized lines of corn and of crosses between them. USDA Bull 1354

Roff DA, Mousseau TA (1987) Quantitative genetics and fitness: lessons from *Drosophila*. Heredity 58:103–118

Rumball W (1974) The decrease in heterozygosity produced by close inbreeding. PhD thesis, Macquarie University

Ryan TA, Joiner BL, Ryan BF (1985) Minitab student handbook, 2nd edn. PWS-Kent, Boston

Shultz FT (1953) Concurrent inbreeding and selection in domestic fowl. Heredity 7:1–21.

Templeton AR, Read B (1983) The elimination of inbreeding depression in a captive herd of Speke's gazelle. In: Schonewald-Cox CM, Chambers SM, MacBryde B, Thomas WL (eds) Genetics and conservation. Benjamin/Cummings, Menlo Park, California, pp 241–261

Wright S (1977) Evolution and the genetics of populations, vol 3, experimental results and evolutionary deductions. Univ. Chicago Press, Chicago