Reduced Genetic Load Revealed by Slow Inbreeding in *Drosophila melanogaster*

B. D. H. Latter, J. C. Mulley,¹ D. Reid² and L. Pascoe³

School of Biological Sciences and Faculty of Agriculture, University of Sydney, Sydney, Australia

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ABSTRACT

The rate of decline in reproductive fitness in populations of *Drosophila melanogaster* inbred at an initial rate of ~1% per generation has been investigated under both competitive and noncompetitive conditions. Breeding population size was variable in the inbred lines with an estimated harmonic mean of 66.7 ± 2.2 . Of the 60 lines maintained without reserves, 75% survived a period of 210 generations of slow inbreeding and were then rapidly inbred by full-sib mating to near-homozygosity. The initial rate of inbreeding was estimated to be $0.96 \pm 0.16\%$ per generation, corresponding to an effective population size of ~50. However, the rate of inbreeding declined significantly with time to average only $0.52 \pm 0.08\%$ per generation over the 210 generation period, most likely due to associative overdominance built up by genetic sampling and selection in the small populations. The total inbreeding depression in fitness was estimated to be $87 \pm 3\%$ for competitive ability and $27 \pm 5\%$ for fitness under uncrowded conditions, corresponding to rates of decline of 2.0 ± 0.3 and $0.32 \pm 0.07\%$, respectively, per 1% increase in the inbreeding coefficient. The frequency of lethal second chromosomes in the resultant near-homozygous lines was of the order of 5%, lethal free second chromosomes showed a mean viability under both crowded and uncrowded conditions of ~95%, and their population cage fitness was 60% that of *Cy/+* heterozygotes. It can be concluded that homozygous genotypes from which deleterious genes of major effect have been eliminated during slow inbreeding may show far less depression in reproductive fitness than suggested by earlier studies of wild chromosome homozygotes. The loss in fitness due to homozygosity throughout the entire genome may be as little as 85–90% under competitive conditions, and 25–30% in an optimal environment.

IT is well known that close inbreeding in animals and outbreeding species of plants usually has deleterious effects on traits related to reproductive fitness and that hybrids in self-fertilized species are frequently superior to their parents in vegetative vigour and the production of fruit and seed (FALCONER 1989; SIMMONDS 1979). The weight of evidence suggests that inbreeding depression and heterosis in outbreeding species are due mainly to partial dominance rather than to heterozygote superiority at individual loci (CROW 1993). Studies of heterosis in self-fertilized species have in addition shown that homozygous lines with yield levels close to that of the parental hybrid may frequently be derived by repeated self-fertilization (UDDIN *et al.* 1994a,b).

Quantitative traits have been shown to differ markedly in the extent to which they are affected by inbreeding, abdominal bristle number in *Drosophila melanogaster* showing no appreciable change in mean under full-sib mating (RASMUSON 1952; KIDWELL and KIDWELL 1966), whereas litter size in mice and yield of grain in maize

are subject to inbreeding depression of the order of 0.7% per 1% increase in the inbreeding coefficient *F* (FALCONER 1989). Under competitive conditions, reproductive fitness has been shown to be reduced by 2.9% per 1% increase in *F* due to continued full-sib mating without reserves in lines derived from a large cage population of *D. melanogaster* (LATTER and ROBERTSON 1962). The inbreeding coefficient in these studies of close inbreeding has been inferred from the mating system involved, assuming the influence of natural selection during inbreeding to be negligible. However, a recent report by RUMBALL *et al.* (1994) has shown that the decline in heterozygosity in *D. melanogaster* under full-sib and double first-cousin inbreeding with reserve matings is only ~80% of that expected, leading to an underestimation of the extent of inbreeding depression.

Chromosome homozygotes can readily be synthesized by the use of special techniques in some species of *Drosophila*, leading to an appreciable reduction in viability and fecundity (LEWONTIN 1974). Approximately 25% of homozygotes for chromosome 2 in *D. melanogaster* are lethal or semilethal, and the remaining "quasi-normal" chromosomes have a mean homozygote viability of ~80% that of random heterozygotes (GREENBERG and CROW 1960; SVED 1971). The mean fecundity of females homozygous for chromosome 2 in

Corresponding author: B. D. H. Latter, 43 Camerons Rd., McLeans Ridges, Lismore N.S.W. 2480, Australia.

¹ Present address: Dept. of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, North Adelaide 5006, Australia.

² Present address: 24 Jellicoe St., Linden Park, S.A. 5065, Australia.

³ Present address: INSERM Unité 36, Collège de France, 3 rue d'Ulm, 75005 Paris, France.

D. pseudoobscura is ~80% that of random heterozygotes (MARINKOVIC 1967). Under the extreme conditions of competition in small population cages, the total fitness of nonlethal second or third chromosome homozygotes in *D. melanogaster* is reduced to roughly 15% that of random heterozygotes (SVED 1971; MACKAY 1985).

Little information is available about the effects of slow inbreeding in cross-fertilized species at a rate of one or a few percent per generation, where the effects of natural selection may be important and direct estimates rather than theoretical predictions of the level of inbreeding are required. Breed or variety crosses in poultry, pigs, sheep, cattle and maize frequently show high levels of heterosis for productive traits related to reproduction and survival, which has been interpreted as due primarily to the inevitable inbreeding entailed in the development of the populations concerned (FALCONER 1989; ROBINSON and COMSTOCK 1955). The level of heterosis exhibited by such between-strain crosses may be quite dramatic, exceeding 25% for yield in maize (ROBINSON *et al.* 1958; MOLL *et al.* 1965) and reaching as high as 40% for fertility in sheep (SIDWELL *et al.* 1962).

In a study of the effects of inbreeding at different rates in *D. melanogaster*, TANTAWY and REEVE (1956) found that the heritability of wing length declined much less rapidly than expected on the basis of the calculated values of the inbreeding coefficient. They suggested that natural selection against homozygotes at some loci, and increasing sensitivity to the effects of further homozygosis as inbreeding proceeds, were both responsible for the reduction in the effective rate of inbreeding.

The effects of artificial selection on litter size in mice were reported by FALCONER (1960), relative to an unselected control line initiated from the same random mating base population and subject to inbreeding at a predicted rate of 1.25% per generation. No change in mean litter size was detected in the control line over a period of 31 generations, despite the observations that rapid inbreeding caused substantial depression in litter size in lines initiated from the same base population (BOWMAN and FALCONER 1960; ROBERTS 1960). LATTER and ROBERTSON (1962) have shown that two slowly inbred control lines of *D. melanogaster* maintained for 25 generations by the transfer of 10 pairs of randomly chosen parents each generation (predicted $\Delta F = 4.4\%$) were reduced in competitive ability by only 0.7% per 1% increase in F , compared with 2.9% under rapid inbreeding. However the lack of adequate replication in these studies makes it impossible to draw general inferences concerning the rate of change of traits related to fitness under slow inbreeding.

The aim of the present investigation was to estimate the rate of inbreeding depression in several measures of reproductive fitness under both competitive and

noncompetitive conditions, in populations of *D. melanogaster* inbred at an initial rate of ~1% per generation. Two wild-derived populations and a long established laboratory cage population were studied, the level of inbreeding achieved being measured directly from the variance in gene frequency among replicate subpopulations for electrophoretic markers. The slow adaptation of the subpopulations to the laboratory environment has been documented by LATTER and MULLEY (1995), and this paper is concerned with comparisons between the inbred subpopulations and the corresponding panmictic populations formed by hybridization and subsequent random mating.

MATERIALS AND METHODS

Details of the origin of the experimental populations, the maintenance of laboratory stocks, the measurement of competitive ability and of noncompetitive fitness and the basic methods of statistical analysis have been given by LATTER and MULLEY (1995). Only a brief summary of this information will be presented here, together with further particulars relevant to the measurement and analysis of the rate of inbreeding and the study of the full extent of inbreeding depression.

The effects of slow inbreeding have been examined in three base populations, one derived from a large laboratory cage population (Canberra) and two recently sampled from the wild (Letona and Tyrrells). Inbreeding was initiated in May 1971 after the samples from the Canberra cage population and the Letona and Tyrrells wild populations and had been kept in the laboratory for 24, 11 and 4 generations, respectively, as large 20-bottle populations, each with roughly 1000 breeding individuals per generation. A set of 20 isolated subpopulations was started from each of the three base populations, designated CI(n), LI(n) and TI(n) for the Canberra, Letona and Tyrrells inbred lines, respectively, where n denotes the number of generation of inbreeding. Each subpopulation was maintained at $25 \pm 2^\circ$ without reserves, by the synchronous mass transfer of parents to a single half-pint bottle each generation. The number of parents per bottle was variable, and periodic counts were made after the 3-day egg laying period to determine the actual distribution of parental population size.

After 210 generations of slow inbreeding, single pair matings within each of the surviving 18 Canberra and 16 Tyrrells subpopulations were used to initiate lines that were rapidly inbred by full-sib mating with reserve cultures for a period of 50 generations. The resulting full-sib lines are designated CIFS(n) and TIFS(n), respectively, where n denotes the number of generations of full-sib mating (Table 1). The subpopulations CI(210) and TI(210) were also slowly inbred for a further 100 generations using the same routine as before.

Panmictic populations CP(80, n), LP(120, n), TP(120, n) and TP(171, n) were formed by intermixing the surviving partially inbred subpopulations within each set after 80, 120, 120 and 171 generations, respectively, followed by n generations of mass mating (Table 1). A second Canberra panmictic population CP(FS, n) was formed by randomly intercrossing 16 of the full-sib inbred lines CIFS(18) in pairs, and progressive intercrossing of the resultant hybrids to form a single multiple-cross, followed by n generations of mass mating. A third Tyrrells panmictic population TP(FS, n) was formed from the TIFS(18) lines in the same manner. Each panmictic population was maintained as a 10-bottle population with periodic intermixing (LATTER and MULLEY 1995).

TABLE 1

Explanation of symbols used to denote the history of inbred, panmictic and wild populations

Designation ^a	History of population
CI(<i>n</i>)	Small subpopulations initiated from the Canberra cage population, and slowly inbred for <i>n</i> generations.
CIFS(<i>n</i>)	Inbred lines derived by <i>n</i> generations of full-sib mating from the CI(210) inbred subpopulations.
CP(<i>m, n</i>)	A large panmictic population initiated by intermixing the small CI(<i>m</i>) subpopulations and random mating for <i>n</i> generations.
CP(FS, <i>n</i>)	A large panmictic population formed by intercrossing the CIFS(18) full-sib inbred lines and random mating for <i>n</i> generations.
CW(<i>n</i>)	A large population resampled from the wild, and random mated in the laboratory for <i>n</i> generations.

^a The first letter denotes the Canberra series of populations, and I, FS, P, W denote inbred, full-sib, panmictic and wild populations, respectively. The Letona and Tyrrells populations have similar designations, with L and T, respectively, replacing the first letter C. The LI(*n*) and TI(*n*) slowly inbred lines were initiated from recently sampled wild populations.

The 45 subpopulations surviving the period of 210 generations of slow inbreeding were surveyed for the presence of chromosome inversions, using standard procedures for examination of the salivary gland polytene chromosomes (LEFEVRE 1976). Larvae were dissected in 0.7% insect saline, and the salivary glands were held in 45% acetic acid for 10–15 sec before staining in a solution prepared by dissolving 5 g Gurr's natural orcein in a mixture of 50 ml glacial acetic acid and 50 ml glacial lactic acid.

The main test of competitive ability used in this study is a modification of the single generation female test of LATTER and ROBERTSON (1962), repeated over a number of generations to assess the repeatability of the estimate of competitive ability relative to the standard mutant competitor stock. Non-competitive fitness was assessed as the biomass or number of offspring produced by a single female laying in a 4 × 1 in. vial. Precise details of these experimental procedures are given by LATTER and MULLEY (1995). Second chromosome homozygotes from the full-sib-mated lines have in addition been tested for viability under crowded and uncrowded conditions using the standard *Cy/Pm* technique and for competitive ability using the population cage balancer equilibration test of SVED and AYALA (1970). The *Cy/Pm* stock was that described by WILTON *et al.* (1989), which is classified as a P strain in the P-M hybrid dysgenesis system.

RESULTS

Census population size: The numbers of male and female parents transferred each generation in the maintenance of the inbred subpopulations CI(*n*), LI(*n*) and TI(*n*) were regulated only by general inspection to avoid excessive overcrowding, and the actual breeding

population size was therefore a random variable. Periodic counts of the number of parents surviving the 3-day egg laying period were made to provide an accurate picture of the distribution of population size N_h , calculated as twice the harmonic mean of the number of male and female parents.

The values of N_h varied widely among individual cultures, with a range from 10 to 191 and a harmonic mean of 66.7 ± 2.2 , based on a sample of 680 counts. The distribution of values of N_h was close to lognormal, with mean 4.26 and SD 0.34 on the scale of natural logarithms. The geometric mean value of $[1 - (2N_h)^{-1}]$ was also calculated for the sample, but was virtually identical to the harmonic mean of N_h . A consequence of the experimental procedure is that the subpopulations were subject to occasional bottlenecks in population size, and their behavior may possibly be different from populations of constant size inbred at the same average rate, especially as regards the possibility of extinction.

The ratio of effective to census breeding population size in bottle cultures of *Drosophila* has been reported to be ~ 0.7 (KERR and WRIGHT 1954a,b; WRIGHT and KERR 1954; CROW and MORTON 1955; BURI 1956). The effective population size in the slowly inbred subpopulations of the present study is therefore expected to be roughly 50, corresponding to a predicted rate of inbreeding of 1% per generation.

Rate of extinction: Of the 60 subpopulations, all survived the first 40 generations of slow inbreeding, 50 survived to generation 120, and 45 to generation 210. The extinction of lines appears not to have been entirely random, significantly more losses having occurred in the Letona lines than in the other two sets of subpopulations. The overall rate of extinction corresponds to a chance of roughly 1 in 800 that a population might be lost in any given generation, possibly due to a severe bottleneck involving the transfer of very few or no female parents. No line was lost by accident, and lines that failed to reproduce gave no advance indication of drastically reduced fertility.

Segregation of chromosome inversions: In the 45 subpopulations surviving to generation 210 no inverted sequences were detected for chromosome 1, but inversions were found in all four arms of the major autosomes. The inversion frequencies were low in all cases except for a terminal inversion in chromosome 2R, which was found at a frequency of almost 25% in the Letona subpopulations (Table 2). The frequency of inversions in the Canberra subpopulations was extremely low by comparison with the wild-derived Letona and Tyrrells subpopulations. Less than 2% of the 368 sets of chromosomes examined in the Canberra lines showed an inverted sequence, and the inversion concerned was not found in either the Letona or Tyrrells lines. There is clear evidence from these observations

TABLE 2

Frequency of chromosome inversions in the Canberra, Letona and Tyrrells sub-populations after 210 generations of slow inbreeding

Chromosome	Breakpoints	Frequency (%)		
		CI(210)	LI(210)	TI(210)
2L	22D;34A	—	—	1.05
2R	52D;60F	—	24.34	3.50
	52C;57A	—	2.21	—
	50B;56D	—	1.77	0.35
	44A;49A	—	—	0.35
3L	63C;72E	—	0.88	1.05
	63B;65A	—	—	0.35
3R	89C;96A	—	1.33	1.75
	90F;94C	—	0.88	—
	93E;98A	1.90	—	—
Total frequency (%)		1.90	31.41	8.39
Haploid sets scored		368	226	286

that very little if any genetic exchange has taken place between sets of subpopulations during the process of slow inbreeding.

Of particular importance is the finding that inversions located near or including the electrophoretic marker loci *Adh* and *Est-6* were very low in frequency in all sets of lines. *Adh* is located on chromosome 2L in the vicinity of subdivision 35B (LINDSLEY and GRELL 1968), and inversions in this region were absent from populations CI(210) and LI(210). An inversion with breakpoints at 22D;34A, presumably *In*(2L)t, occurred with a frequency of roughly 1% in TI(210). *Est-6* is located on chromosome 3L between divisions 66 and 70, inversions in this vicinity being absent in CI(210), and occurring at a frequency of the order of 1–1.5% in LI(210) and TI(210). The drift in allele frequencies at the *Adh* and *Est-6* loci during slow inbreeding is therefore expected to have been virtually unaffected by the segregation of chromosome inversions.

Rate of inbreeding: Estimates of the inbreeding coefficient have been made separately for each of the three sets of subpopulations throughout the 210 generations of slow inbreeding, based on the variance in gene frequency among replicate subpopulations for the fast alleles at the *Adh* and *Est-6* loci. Other *Est-6* variants were detected at low frequency in the wild-derived Letona and Tyrrells populations (FRANKLIN 1981), but these provide little additional information on the rate of inbreeding.

The value of the inbreeding coefficient F_t has been calculated as

$$F_t = (F^* - 1/2r) / [(1 - 1/2r) + F^*/k] \quad (1)$$

with

$$F^* = s^2 / [p(1 - p)] \quad (2)$$

$$= [\sum p_i^2 - kp^2] / [(k - 1)p(1 - p)] \quad (3)$$

where p is the mean and s^2 the variance of the gene frequencies p_i in the k replicate subpopulations based on a sample size of r individuals per subpopulation. Sample sizes of at least 60 individuals per subpopulation were used in the early stages of the experiment, and 40 or more after generation 68. This procedure estimates the inbreeding coefficient of the inbred lines relative to the conceptual panmictic population formed by intermating an infinite set of such lines. Apart from the approximate correction for sampling error, Equation 1 corresponds to Equation 19 of NEI (1976).

The inbreeding coefficients F_t estimated from *Adh* and *Est-6* gene frequencies do not differ significantly (Table 3), and means over the two loci have therefore been plotted in Figure 1. The initial average rate of inbreeding for the Canberra, Letona and Tyrrells lines given by the electrophoretic data in generations 19, 35 and 26, respectively, is $0.96 \pm 0.16\%$ per generation, based on the mean value of $(-1/t) \log_e (1 - F_t)$, where F_t is the inbreeding coefficient in generation t . This corresponds to an effective population size of $N_e = 52$. By contrast, the final estimates of F for the three sets of lines correspond to an average rate of inbreeding of $0.52 \pm 0.08\%$ per generation, which is significantly different from the estimated initial rate and suggests an average effective population size over the 210 generation period of $N_e = 96$. Using all 20 available estimates of F from Table 3, the linear regression of $(-1/t) \log_e (1 - F_t)$ on t has intercept $a = 0.0105 \pm 0.0012$ and slope $b = -0.000028 \pm 0.000008$, indicating a highly significant reduction in the rate of inbreeding throughout the 210 generation period ($P = 0.002$). The separate regression coefficients for the Canberra and Tyrrells populations were significantly negative ($P = 0.008$ and 0.024 , respectively) but that for the Letona population did not differ significantly from zero.

As a first approximation, let us suppose that the value of ΔF changes by a constant multiple $1 - \delta$ each generation, *i.e.*,

$$\Delta F_t = \Delta F_1 (1 - \delta)^{t-1} \quad (4)$$

and

$$F_t = 1 - \prod_i (1 - \Delta F_i) \quad (5)$$

Fitting Equations 4 and 5 to the data points of Table 3 leads to least squares estimates of $\delta = 0.0090$ and $\Delta F_1 = 0.0101$, and a residual SD of 0.118 with 17 d.f. The curve in Figure 1 is based on these estimates and gives a satisfactory empirical fit to the data points. However, it should be emphasized that Equation 4 has been chosen for simplicity to accommodate the fact that the rate of inbreeding declines with time and does not correspond to known population genetics theory.

TABLE 3

Estimates of the inbreeding coefficient from the variance in allozyme frequencies among replicate sub-populations

Generation	Population	k^a	Adh			Est-6			Mean F_i
			p	s^2	F_i	p	s^2	F_i	
19	CI	20	0.58	0.055	0.22	0.14	0.030	0.24	0.23
26	TI	20	0.69	0.054	0.24	0.30	0.037	0.17	0.20
35	LI	20	0.80	0.043	0.26	0.17	0.020	0.13	0.20
68	CI	20	0.64	0.138	0.58	0.15	0.043	0.33	0.46
163	CI	18	0.67	0.136	0.58	0.09	0.040	0.48	0.53
170	LI	12	0.75	0.151	0.74	0.16	0.073	0.52	0.63
172	TI	16	0.73	0.091	0.44	0.25	0.114	0.57	0.51
207	TI	16	0.77	0.081	0.44	0.20	0.105	0.63	0.53
210	CI	18	0.62	0.182	0.74	0.11	0.049	0.50	0.62
210	LI	11	0.69	0.193	0.83	0.15	0.094	0.69	0.76

The frequency of the fast allele at the Adh or Est-6 locus is denoted by p , the variance in allele frequency by s^2 and the estimated inbreeding coefficient corrected for sampling error by F_i .

^a Number of surviving slowly inbred lines.

For purposes of computation, a satisfactory approximation to the curve specified by Equations 4 and 5 is given by

$$F_t = 1 - \exp \{-\Delta F_i [1 - (1 - \delta)^t] / \delta\} \quad (6)$$

For the estimated values of δ and ΔF_i , this formula is adequate for at least 500 generations.

Decline in reproductive fitness with inbreeding: LATTER and MULLEY (1995) have presented estimates of the competitive ability of the Canberra, Letona and Tyrrells subpopulations after more than 120 genera-

tions of slow inbreeding and the corresponding panmictic populations formed by intermixing the lines within sets and random mating for an extended period. The competitive indices were based on a modification of the single generation female test of LATTER and ROBERTSON (1962), under conditions of competition approximating those experienced during the process of slow inbreeding. Further tests of the same type were made for the near-homozygous lines CIFS(30) and TIFS(30) after a period of 30 generations of full-sib mating with reserve cultures to avoid loss of lines, commencing with the subpopulations CI(210) and TI(210). The panmictic populations used for comparison were CP(FS,8) and TP(FS,8), formed by intercrossing the full-sib inbred lines and random mating for an average of eight generations.

Table 4 sets out the complete set of relative competitive indices (RCI), expressed as a fraction of the competitive index of the panmictic population in each instance, from tests involving full feeding of the adult competing individuals (LATTER and MULLEY 1995). The estimated inbreeding coefficients F_i for the inbred lines are based on the curve of best fit in Figure 1 using Equations 4 and 5. The inbreeding coefficient of a panmictic population $P(m,n)$, based on the intermixture of k inbred lines at generation m , has been estimated from the value of F_i in generation m as F_m/k , followed by inbreeding for n generations at a rate of 0.1% per generation, i.e., $1 - (1 - F_m/k)(0.999^n)$. Two sets of RCI values are presented in Table 4, calculated from the observed number or weight of offspring produced from the competition cultures after 2 days and after 10 days of emergence. The SEs of the ratios have been calculated using the formulas of LATTER and MULLEY (1995). Table 5 provides a similar set of data derived from comparisons of reproductive fitness in the absence of com-

FIGURE 1.—Mean inbreeding coefficients F_i estimated from Adh and Est-6 gene frequencies in replicate subpopulations (Table 3). The solid curve is the expected values of F assuming a constant rate of inbreeding of 0.96% per generation; the dashed curve is based on Equations 4 and 5 using least squares estimates of $\delta = 0.0090$ and $\Delta F_i = 0.0101$.

TABLE 4
Competitive indices of inbred relative to outbred populations
with full feeding of competing adult individuals

Comparison ^a	F_I^b	F_P^b	Relative Competitive index (RCI)	
			2 days emergence	10 days emergence
CI(120) <i>vs.</i> CP(80,40) ^c	0.53	0.06	0.50 ± 0.09	0.66 ± 0.09
CI(157) <i>vs.</i> CP(80,77) ^c	0.57	0.10	0.37 ± 0.09	0.73 ± 0.08
LI (163) <i>vs.</i> LP(120,43) ^c	0.58	0.07	0.47 ± 0.08	0.39 ± 0.03
TI(187) <i>vs.</i> TP(171,16) ^c	0.60	0.05	0.18 ± 0.03	0.38 ± 0.03
CIFS(30) <i>vs.</i> CP(FS,8) ^d	1.00	0.07	0.17 ± 0.03	0.25 ± 0.04
TIFS(30) <i>vs.</i> TP(FS,8) ^d	1.00	0.07	0.16 ± 0.04	0.23 ± 0.06
Mean rate of decline			exp[(-2.01 ± 0.25)F]	exp[(-1.37 ± 0.20)F]

^a The comparison of CI(120) and CP(80,40) involved CyL/P_m and yscw stocks as standard competitors. All other comparisons used Canysc as standard.

^b Inbreeding coefficients for the inbred (F_I) and panmictic (F_P) populations estimated from the curve of Figure 1.

^c Based on the numbers of wild-type and mutant offspring produced in competition cultures.

^d Based on the total weight of wild-type and mutant offspring from competition cultures.

petition (RF), *i.e.*, number or weight of offspring produced by single females under uncrowded conditions.

The regressions of $\log_e$ (RCI) on $F_I - F_P$ in Table 4, and those for $\log_e$ (RF) in Table 5, all have intercepts that do not differ significantly from zero, indicating no conspicuous departure from linearity, though the test is not one of great accuracy. The mean rates of decline of RCI as a function of F have therefore been calculated by averaging the individual values of $\log_e$ (RCI) / ($F_I - F_P$) in Table 4, with SE based on their repeatability over the separate experiments. Similar calculations were made of the mean values of $\log_e$ (RF) / ($F_I - F_P$) in Table 5. It should be noted that the estimates of RCI in Table 4 are expected to overestimate the extent of inbreeding

depression to the extent that adaptation in the Letona and Tyrrells panmictic populations proceeds more rapidly than in the corresponding inbred lines (LATTER and MULLEY 1995). However, the extent of this bias is trivial for the estimates of RCI obtained from the CIFS(30) and TIFS(30) populations at near-homozygosity.

Figure 2 shows the individual points, and the mean rate of decline as a function of the inbreeding coefficient for relative fitness under noncompetitive conditions and for the relative competitive index based on 2 days emergence with a premium therefore placed on early emergence from the competition culture. The curves indicate a total inbred load of $27 \pm 5\%$ under

TABLE 5
Noncompetitive reproductive fitness of inbred relative to outbred populations

Comparison	F_I^a	F_P^a	Relative noncompetitive fitness (RF)	
			1 day emergence	8 days emergence
CI(130) <i>vs.</i> CP(80,50) ^b	0.54	0.07	0.91 ± 0.03	0.98 ± 0.02
LI(143) <i>vs.</i> LP(120,23) ^b	0.56	0.06	0.76 ± 0.05	0.94 ± 0.03
LI(143) <i>vs.</i> LP(120,23) ^c	0.56	0.06	0.89 ± 0.07	0.88 ± 0.04
TI(144) <i>vs.</i> TP(120,24) ^b	0.56	0.06	0.83 ± 0.03	0.71 ± 0.05
TI(144) <i>vs.</i> TP(120,24) ^c	0.56	0.06	0.76 ± 0.04	0.84 ± 0.03
CI(177) <i>vs.</i> CP(80,97) ^c	0.59	0.12	0.82 ± 0.03	0.80 ± 0.03
CIFS(10) <i>vs.</i> Hybrids ^d	0.96	0.00	0.73 ± 0.08	0.76 ± 0.03
TIFS(10) <i>vs.</i> Hybrids ^d	0.96	0.00	0.66 ± 0.11	0.73 ± 0.10
Mean rate of decline			exp[(-0.39 ± 0.05)F]	exp[(-0.32 ± 0.07)F]

^a Inbreeding coefficients for the inbred (F_I) and panmictic (F_P) populations estimated from the curve of Figure 1.

^b Mean number of progeny produced by single pairs in 4 × 1-in. vials.

^c Mean number of progeny produced by 2, 5 and 10 pairs of parents in 1/4 pint bottles.

^d Total weight of progeny produced by single pairs of inbred or F_I hybrid parents in 4 × 1-in. vials. The offspring of the hybrid parents are F_2 individuals rather than random heterozygotes.

FIGURE 2.—The decline in relative fitness as a function of the difference in inbreeding coefficient ($F_i - F_p$) between the inbred and panmictic populations. The points are estimates of relative fitness under noncompetitive conditions (RF) after eight days emergence (Table 5) with curve of best fit $RF = \exp(-0.32F)$, and the relative competitive index (RCI) after 2 days emergence (Table 4), with the corresponding curve $RCI = \exp(-2.01F)$.

noncompetitive conditions and $87 \pm 3\%$ under the competitive regime.

The fitness of chromosome homozygotes: A sample of 127 second chromosomes was extracted from 10 of the CIFS(43) and 7 of the TIFS(43) near-homozygous lines, and the viability of chromosome homozygotes assessed by the standard Curly Plum technique (WALLACE 1956). The frequency of lethal or near-lethal chromosomes, *i.e.*, those with estimated homozygous viability $< 10\%$, was 7/127 or 5.5%, and the average viability of the remaining homozygotes was 0.94 ± 0.03 , with no significant difference between the Canberra and Tyrrells lines.

A set of 12 chromosomes with apparently above average estimates of viability (six from each of the Canberra and Tyrrells lines) was chosen for homozygous fitness determination by the balancer equilibration test of SVED and AYALA (1970). The mean of the initial viability estimates for the chosen chromosomes was 1.22 relative to $Cy/+$. However, the viability ratio test for 11 of the chromosomes that reached stable frequencies in the population cages gave a mean of 0.94 ± 0.02 , indicating little effect of selection based on the initial viability estimate. A further viability test of the same chromosomes under crowded larval conditions produced a mean of 0.96 ± 0.02 , with no correlation between the two tests ($r = -0.12$; 9 d.f.).

The initial frequency of $Cy/+$ individuals in the cage populations was close to 66%, produced by a $Cy/+ \times$

TABLE 6

Fitness estimates for individual second chromosomes given by the balancer equilibration test of SVED and AYALA (1970)

Chromosome	Frequency $Cy/+^a$ (h)	Viability (v)	Fitness ^b (w)
Canberra			
1	0.38	0.99	0.70
2	0.44	0.94	0.63
3	0.52	1.06	0.44
4	0.32	1.00	0.77
5	0.46	0.96	0.60
6	0.35	0.95	0.75
Tyrrells			
1	0.55	0.91	0.45
2	0.48	0.97	0.55
3	0.50	0.76	0.62
4	0.52	0.87	0.53
5	0.47	0.89	0.60
Mean $\pm$ SE	0.45 ± 0.02	0.94 ± 0.02	0.60 ± 0.03

^a The frequency of $Cy/+$ adults in cage samples.

^b Calculated as $w = 1 - \frac{1}{2}hv/(1 - h)$.

$Cy/+$ cross for each chromosome. In one test the frequency of Cy was reduced to zero, possibly due to contamination of the cage population. In the remaining 11 cages stable frequencies of $Cy/+$ adults were reached when their frequency had been reduced to between 32 and 55%. Details of the estimates of fitness relative to $Cy/+$ for these 11 chromosomes are given in Table 6.

The sample of inbred line chromosomes had a mean viability of 0.94 ± 0.02 and cage fitness of 0.60 ± 0.03 relative to $Cy/+$ heterozygotes, with little difference between the Canberra and Tyrrells chromosomes. (The mean fitness of 0.60 ± 0.03 may be an underestimate if the rejected 12th chromosome was one of high homozygous fitness.) These values can be compared with the estimates obtained by SVED (1971) of 0.73 ± 0.05 for viability and 0.28 ± 0.08 for mean fitness, using a random set of 24 nonlethal wild chromosomes from wineries located within a few miles of each other, including the Tyrrells winery. There is therefore clear evidence that natural selection during the history of the populations in the laboratory has produced a dramatic improvement in both mean homozygote viability and homozygous cage fitness relative to $Cy/+$ heterozygotes, in addition to a reduction in the frequency of lethal bearing chromosomes.

Effects of inbreeding on quantitative traits: Table 7 summarizes data on the level of quantitative variation recorded in the slowly inbred subpopulations from generations 160 to 315, relative to that in the corresponding panmictic populations. The traits scored were the sum of abdominal bristle numbers on the 4th and 5th sternites and the sum of sternopleural bristle numbers

TABLE 7

The magnitude of $\sigma_p^2 - \sigma_d^2$ for abdominal and sternopleural bristle number in inbred subpopulations relative to the corresponding panmictic population^a

Mean generations of inbreeding	Populations ^b	Relative magnitude of $\sigma_p^2 - \sigma_d^2$		
		Abdominals	Sternopleurals	Mean
160	CI/CP	0.44 ± 0.11	—	0.42 ± 0.08
	LI/LP	0.39 ± 0.12	—	
205	CI/CP	—	0.69 ± 0.12	0.51 ± 0.07
	LI/LP	—	0.52 ± 0.13	
	TI/TP	—	0.32 ± 0.09	
304	CI/CP	0.52 ± 0.08	0.50 ± 0.10	0.48 ± 0.04
	TI/TP	0.57 ± 0.10	0.32 ± 0.05	
Mean		0.48 ± 0.05	0.47 ± 0.05	

^a σ_p^2 denotes the within-culture phenotypic variance, and σ_d^2 the within-fly developmental error component calculated from the difference between the two counts on the same individual. SEs are based on the repeatability of the estimates over replicate cultures and generations.

^b Inbred/Panmictic.

on both sides of the fly. The phenotypic variance σ_p^2 , and the variance σ_d^2 of the difference between the two counts on the same individual, were calculated within cultures for each trait, the difference $\sigma_p^2 - \sigma_d^2$ measuring the total genetic variance within populations, together with a small component due to between-fly environmental effects (LATTER 1964).

It is not possible to convert these data into precise estimates of the inbreeding coefficient, because the between-fly environmental component of variance and the extent of nonadditive genetic variance are unknown. However, it is evident from Table 7 that the within-line genetic variance has been reduced by at least 50% in the slowly inbred lines and that changes over the latter half of the 300-generation period are minor by comparison with those in the first 160 generations. These observations support inferences drawn from estimates of the inbreeding coefficient in Table 3 based on electrophoretic data, *i.e.*, that the effective rate of inbreeding has declined appreciably throughout the period surveyed.

DISCUSSION

The observed rate of inbreeding: The initial rate of inbreeding in the Canberra, Letona and Tyrrells subpopulations was estimated from electrophoretic data to be $0.96 \pm 0.16\%$ per generation, corresponding to an effective population size of $N_e = 52$, *i.e.*, roughly 80% of the observed census size of $N_h = 67$. However, the average rate of inbreeding over 210 generations was $0.52 \pm 0.08\%$ per generation, and the linear regression of the mean rate of inbreeding on number of generations was negative and highly significant.

It is possible to account for this decline in rate of inbreeding by a model that does not involve natural

selection if appreciable migration into the slowly inbred lines is assumed each generation, using the equation

$$F_t = (1 - m)^2 \{(2N)^{-1} + [1 - (2N)^{-1}]F_{t-1}\} \quad (7)$$

for the island model with a rate of migration m per generation and effective breeding size N (WRIGHT 1931). The least squares estimates of m and N from the data of Table 3 are $m = 0.0027$ and $N = 52$ with residual SD of 0.119, corresponding to an average rate of migration of $Nm = 0.14$ individuals into each subpopulation. This implies that 6–8 of the slowly inbred lines were contaminated each generation, which is far too high to be accepted. Objective evidence that the migration model is unacceptable is provided by the data for chromosome inversion frequencies presented in Table 2. All three sets of subpopulations were maintained together and transferred at the same time, so that lack of cross contamination between sets implies the same within sets.

It can be concluded that natural selection is responsible for the reduction in the rate of inbreeding in the slowly inbred subpopulations, as measured by the drift in gene frequency at the *Adh* and *Est-6* loci (Table 3), and the reduction in between-fly variance of abdominal and sternopleural bristle number (Table 7). Selection in favor of chromosome inversion heterozygotes has apparently played an insignificant role in opposing the changes in gene frequency due to inbreeding at the *Adh* and *Est-6* loci (Table 2).

It can be shown by computer simulation that the hypothesis of single-locus overdominance at the *Adh* and *Est-6* loci does not adequately account for the data of Table 3. With initial equilibrium frequencies of the less frequent allele of 0.1–0.2, the progressive reduction in mean gene frequency predicted by ROBERTSON (1962) can be shown to be far greater, and the retarda-

tion of the rate of loss of heterozygosity considerably less, than that observed for Letona Adh and Est-6, and Canberra Est-6.

There is little doubt that models involving gene frequency-dependent selection could be proposed which would be compatible with these data, with an initial rate of loss of heterozygosity due almost entirely to genetic drift, but strong opposition to further inbreeding as gene frequencies approach fixation. There is some evidence in favour of this hypothesis for Est-6 which has been reviewed by LEWONTIN (1974), but it seems unlikely that the same model would apply to the many genes contributing to variation in abdominal and sternopleural bristle number, which have been shown by MACKAY (1985) to be weakly associated with reproductive fitness. Indeed it is improbable that the direct effects of natural selection on these quantitative trait loci are responsible for the retardation in the rate of inbreeding evident in Table 7.

The intense natural selection detected in this study at electrophoretic and quantitative trait loci is most likely due to associative overdominance built up over the generations by genetic sampling and selection in the slowly inbred populations, due to the presence of deleterious partially recessive mutants in the regions neighboring the marker loci. A similar conclusion was reached by RUMBALL *et al.* (1994) for the case of inbreeding by continued full-sib or double first-cousin matings. Multi-locus computer simulation studies are necessary to determine the range of values of the parameters in such genetic models which give satisfactory agreement with the experimental observations of rates of inbreeding and reduction in reproductive fitness.

Extent of heterosis revealed by slow inbreeding: The data of Tables 4 and 5 suggest that the decline in fitness as a function of the inbreeding coefficient is linear when fitness is measured on a logarithmic scale, *i.e.*, that the genes concerned with inbreeding depression or heterosis are acting multiplicatively. LATTER and ROBERTSON (1962) also observed an essentially linear decline in competitive index on a logarithmic scale in their study of the effects of rapid inbreeding by full-sib mating in an established laboratory cage population of *D. melanogaster*.

The objective of this study was to assess the full extent of heterosis or inbreeding depression due to homozygosity throughout the entire genome, when the opportunity is given for deleterious genes of large effect to be eliminated during an extended period of slow inbreeding. All the evidence suggests that the genetic load revealed is similar in magnitude in the wild and cage populations tested. The conclusions are almost identical whether based on the observed rate of decline as a function of F , or on the observed mean fitness of near-homozygous genotypes, *i.e.*, that the total reduction in fitness is of the order of 85–90% for the early

emergence of offspring under competitive conditions (Table 4), and 25–30% under noncompetitive conditions (Table 5).

The inbreeding depression of 2.0% per 1% increase in F observed under competitive conditions is considerably less than the 2.9% per 1% increase in F observed by LATTER and ROBERTSON (1962) for full-sib inbreeding in the Kaduna cage population. The figure for rapid inbreeding is likely to be an underestimate, in view of the finding by RUMBALL *et al.* (1994) that the realised rate of inbreeding under full-sib mating is only 80% of the theoretical prediction. However, the competitive indices were not precisely the same in the two studies, the measurements in the slow inbreeding experiment being based on a test that includes male fertility but not competitive male mating ability (LATTER and MULLEY 1995), while those in the rapid inbreeding experiment made use of the competitive index defined by KNIGHT and ROBERTSON (1957) that overemphasizes the importance of the male fitness component. In addition, the calculation of inbreeding depression by LATTER and ROBERTSON (1962) assumed that lines lost during the process were of zero fitness, leading to a possible overestimation of the rate of decline.

A more direct comparison of the effects of slow *vs.* instantaneous inbreeding is provided by estimates of the fitness of second chromosome homozygotes relative to $Cy/+$ heterozygotes using the balancer equilibration test of SVED and AYALA (1970). It has been noted elsewhere (LATTER and SVED 1994) that this test of fitness under population cage conditions gives somewhat higher estimates of inbreeding depression than the single-generation competitive index, though the difference is not nearly as marked as has been suggested by HAYMER and HARTL (1982). The cage fitness relative to $Cy/+$ of homozygotes for nonlethal chromosomes produced by slow inbreeding (Table 6) is approximately twice that for comparable chromosomes sampled from the wild. The difference between the figure of 0.28 ± 0.08 for homozygous fitness reported by SVED (1971) for recently sampled wild chromosomes, and the value of 0.60 ± 0.03 observed in the present study can be attributed entirely to natural selection in the laboratory, since precisely the same technique and reference Cy chromosome have been used in both experiments.

LATTER and MULLEY (1995) measured the extent of adaptation to laboratory conditions in the cage and bottle populations involved in the present study, showing that the panmictic populations exceeded resampled wild populations by up to 90% in fitness under competitive conditions. If the alleles favoured by natural selection in the laboratory show additive gene action or ambidirectional dominance, we would expect the fitness of second chromosome homozygotes relative to $Cy/+$ to have been increased as a result by roughly 15–20%.

The major part of the improvement in homozygous fitness can therefore be attributed to the reduction in frequency of deleterious genes of large effect during the period of slow inbreeding.

The observations of SVED (1971, 1975) and WILTON and SVED (1979) suggest that the mean fitness of completely homozygous nonlethal genotypes in *Drosophila melanogaster* under the conditions of intense competition in small population cages, should be $\sim 0.15 \times 0.1 \times 0.6 = 0.01$ relative to random heterozygotes. This corresponds to a rate of decline of roughly 4.5% per 1% increase in *F*. The present experiment indicates that if deleterious genes of large effect were eliminated in addition to lethals, homozygous fitness may be as high as 0.04 in a population cage environment and of the order of 0.10–0.15 under the less extreme conditions of crowding involved in the single generation tests of competitive ability.

It is not clear which set of laboratory test conditions comes closest to those prevailing in natural populations, but the single generation tests of competitive ability have been shown by LATTER and MULLEY (1995) to involve complete utilization of the resources available to the competing populations, and these conditions may well correspond to the normal level of crowding experienced by wild populations. The total inbred load under competitive conditions due to genes of minor effect may therefore be considerably less than that suggested by earlier studies of wild chromosome homozygotes.

Fitness under noncompetitive conditions: The data of Table 5 make it clear that the mean fitness of genotypes close to homozygosity throughout the entire genome may be as high as 70–75% that of outbred populations under noncompetitive conditions (Figure 2). The genotypes concerned are the product of an extended period of gradual inbreeding and adaptation to the crowded conditions of mass transfer used to maintain the laboratory populations. For extrapolation to domestic animal and plant populations this figure may be the most important indicator of the unavoidable consequences of inbreeding, bearing in mind that further selection among inbred lines may have identified homozygous genotypes of even higher fitness.

The observed total inbreeding depression of 25–30% is comparable to the levels of heterosis exhibited by between-strain crosses in outbreeding species of plants and animals, but somewhat greater than that shown by hybrids between adapted genotypes of inbreeding species such as wheat (UDDIN *et al.* 1994a). It is considerably less than the estimates of inbreeding depression for litter size in mice and grain yield in maize derived from studies of rapid inbreeding (FALCONER 1989).

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