Decline in Heterozygosity Under Full-Sib and Double First-Cousin Inbreeding in *Drosophila melanogaster*

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ABSTRACT

The effects of inbreeding on heterozygosities and reproductive fitness were determined by carrying out full-sib and double first-cousin inbreeding in *Drosophila melanogaster* populations for up to 18 generations. Parents were scored each generation for five or six polymorphic enzyme loci, and progeny numbers per pair were recorded. Inbreeding depression, in the form of significant reductions in progeny numbers and significant extinction of lines, was observed. Heterozygosity decreased at a significantly slower rate than predicted, being about 80% of expected. The full-sib and double first-cousin treatments showed similar disagreement with expectations over comparable ranges of inbreeding. Natural selection was shown to favor heterozygotes in the inbred lines. Associative overdominance was the most probable explanation for the slower than expected decline in heterozygosity.

INBREEDING is the mating together of individuals related by descent, resulting in chance changes in allelic frequencies within inbred lines, genetic differentiation among lines and an overall decline in heterozygosity. Inbreeding usually reduces reproductive performance and survival, a phenomenon called inbreeding depression (FALCONER 1989).

The degree of inbreeding of an individual is usually measured by WRIGHT's (1921, 1922) inbreeding coefficient F. The expected heterozygosity at a single locus in an inbred populations is given by the well known formula $2pq(1 - F)$ (WRIGHT 1921), where p and q are the allelic frequencies at a diallelic locus and F the inbreeding coefficient. This expression assumes no mutation, gene flow or selection. However, in real populations some genes are likely to be subject to selection, or may be linked to other genes that are under selection. Because individuals with the same expected inbreeding coefficients vary in their degree of homozygosity (see WEIR and COCKERHAM 1973; FRANKLIN 1977), there is ample opportunity for natural selection to modify the array of genotypic frequencies, particularly to favor the more heterozygous individuals.

Despite the widespread application of inbreeding theory to many aspects of population, quantitative and conservation genetics, little is known of the accuracy with which theoretically calculated inbreeding coefficients predict the decline in heterozygosity in populations undergoing systematic inbreeding.

In fact, there is a considerable body of evidence, mainly circumstantial, that the decline in heterozygosity under inbreeding is slower than theory predicts (see ERIKSSON *et al.* 1976; WRIGHT 1977; CONNOR and BELLUCCI 1979; STRAUSS 1986; MITTON 1989; MINA *et al.* 1991). Theoretical work has shown that natural selection favoring heterozygotes can slow the decline in heterozygosity under inbreeding (HAYMAN and MATHER 1953; REEVE 1955). Associative overdominance due to linkage to an overdominant locus (BARTLETT and HALDANE 1935; REEVE and GOWER 1958; COCKERHAM and RAWLINGS 1967; STROBECK 1980) or to loci with deleterious alleles in mutation-selection balance (OHTA 1971; COCKERHAM and RAWLINGS 1967; OHTA and COCKERHAM 1974) can have similar effects. In fact, CHARLESWORTH (1991) showed that a wide range of multilocus models will lead to associative overdominance at neutral marker loci (see DISCUSSION). However, there has been no large scale experimental study of changes in heterozygosity under inbreeding. This theory is used in many practical circumstances, particularly to predict the number of generations of inbreeding required to produce homozygosity; it is important that its predictions be examined experimentally.

The aim of this work was to compare observed with predicted rates of decline in heterozygosity under two different rates of inbreeding and to assess the changes in reproductive fitness in the same lines. *Drosophila melanogaster* populations were inbred for up to 18 generations by full-sib or double first-cousin matings, and the decreases in heterozygosities were measured at six enzyme loci. Estimates were made of the viabilities of genotypes from segregation analyses. Over the course of the experiment, progeny numbers were reduced significantly, and a number of lines became extinct, as expected. Heterozygosity declined at a slower rate than predicted by simple inbreeding theory; the departure

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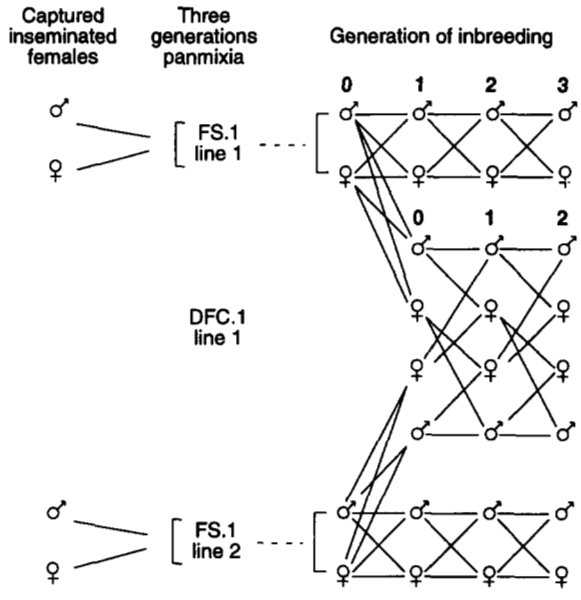

FIGURE 1.—Foundation of the full-sib 1 (FS.1) and double first-cousin 1 (DFC.1) treatments.

from expectations was attributable to natural selection.

MATERIALS AND METHODS

Stocks: Population 1 was founded from 300 wild inseminated *D. melanogaster* females captured at Tyrrell's Vineyards Pty. Ltd. in the Hunter Valley of New South Wales, Australia. These were maintained as isofemale lines (with approximately 60 parents per generation) for three generations prior to founding the FS.1 and DFC.1 treatments that represent the major part of the study (see below).

Population 2 originated from a single female heterozygous for several allozyme loci, captured at the same location. Her descendants were maintained for 20 generations as a laboratory population of approximately 600 flies per generation before the FS.2 treatment was begun. Allele frequencies indicated that the captured female had mated only once, so that population 2 began with four genomes.

Full-sib 1: The first generation of FS.1 (see Figure 1) consisted of 120 pair matings between virgins, one pair from each of 120 randomly chosen population 1 isofemale lines. Each female was assumed to have mated once, resulting in a theoretical inbreeding level of parents in FS.1 of 0.25. FS.1 ran for eighteen consecutive generations of full-sib mating. The inbreeding coefficients for these lines are slightly different from those typically reported for full-sib inbreeding, due to the method used to found them.

Double first-cousin 1: Sixty DFC.1 lines (see Figure 1) were set up using surplus F_1 adults from random pairings of FS.1 lines. In this system two single pair matings in each generation swap one offspring for use as a replacement parent. DFC.1 ran for seventeen consecutive generations at the same time, and under the same conditions, as FS.1. Because the parents of each of the initial matings were unrelated, the initial F value was 0. However, the subsequent inbreeding coefficients for these lines differ slightly from those normally presented for double first-cousin inbreeding, because of the prior inbreeding of the parents.

Full-sib 2: Seventy-eight FS.2 lines were founded from population 2 in a similar manner to the FS.1 lines, except that all lines were founded from the progeny of one pair and the lines were founded after 20 generations, rather than three generations. The F values relevant for inbreeding depression and for drift variances in this base population are different. For assessing inbreeding depression, the base population F was assumed to be 0.25, as there had been one generation of sib-mating. This ensures comparability with FS.1 and DFC.1, and uses the wild population as the base. Conversely, the appropriate F for the heterozygosities is $F = 0$, since the process being followed is a Wahlund drift process. While this population is expected to have a reduced level of heterozygosity compared to the wild population from which it was drawn, the FS.2 base population was a single random mating population, and so had a theoretical inbreeding coefficient of 0. FS.2 was run for eleven generations.

Maintenance of lines: All treatments were run using a fourteen day life cycle at 25 ± 1 °C with single pair matings in vials containing medium F (CLARINGBOLD and BARKER 1961). Virgin females and males were collected and pair matings set up to produce the next generation. To minimize the loss of lines, two reserve pair matings were also set up. These were not used unless the original mating failed. While the use of spares allows natural selection to operate between spare matings within lines, reserve matings are used in almost all practical inbreeding studies (*e.g.*, WALLACE and MADDERN 1965; MINA *et al.* 1991) to avoid excessive loss of lines and to reduce selection among lines.

The genotypes of parents were identified by electrophoresis after allowing five to nine days to reproduce. In FS.1, counts were made of the number of progeny eclosing between the ninth and fourteenth days after setting up a mating.

Electrophoresis: Six autosomal enzyme loci were routinely scored in FS.1 and DFC.1: α-glycerophosphate dehydrogenase-1 (*α-Gpdh*, 2:20.5; EC 1.1.1.8); malate dehydrogenase (*Mdh*, 2:37.2; EC 1.1.1.37); alcohol dehydrogenase (*Adh*, 2:50.1; EC 1.1.1.1); esterase-6 (*Est-6*, 3:36.0; EC 3.1.1.1); octanol dehydrogenase (*Odh*, 3:49.2; EC 1.1.1.73) and aldehyde oxidase (*Aldox*, 3:56.6; EC 1.2.1.3) (O'BRIEN and MACINTYRE, 1978).

The six loci of FS.2 were *Mdh*, *Adh*, *Est-6*, *Odh* and malic enzyme (*Mdh-NADP*, 3:53.1; EC 1.1.1.40) (FRANKLIN and RUMBALL 1971) and NADP-isocitrate dehydrogenase (*Idh-NADP*, 3:27.1; EC 1.1.1.42) (limited data only) (O'BRIEN and MACINTYRE 1978).

There were two alleles segregating at all loci except *Est-6*, which had three common alleles. The $Est\text{-}6^{1.0}$, $Est\text{-}6^{1.1}$ and $Est\text{-}6^{1.25}$ alleles were segregating in FS.2, and in addition $Est\text{-}6^{1.15}$ (FRANKLIN 1971) was segregating in FS.1 and DFC.1. The rare $Est\text{-}6^{1.15}$ allele was grouped with $Est\text{-}6^{1.25}$ in full-sib analyses and both were grouped with $Est\text{-}6^{1.1}$ in double first-cousin analyses to avoid excessively large matrices during analyses.

Electrophoresis was carried out using 8 × 8 × 0.3 cm density gradient polyacrylamide *Gradipore* gels (supplied by Gradient Pty. Ltd., Australia) with a pH 8.9, 0.1 M tris-borate continuous buffer using methods adapted from HUBBY (1963). Gels were run for 2 hr. Several loci of one fly were identified by dividing the fly homogenates between several gels, by slicing gels before staining, and by multiply staining gels for enzymes with non-overlapping mobilities. Staining methods are described by RUMBALL (1974).

Inversions: Populations were surveyed for inversions approximately sixty generations after being sampled for the inbreeding program. Between four and ten F_1 larvae were examined from matings between each of the 26 surviving inbred lines of FS.1 and DFC.1 and a chromosomally normal Ore-RC stock. Two of the lines (7.7%) were segregating for *In (2L)t* (LINDSLEY and GRELL 1968). Three of the 26 lines (11.5%) contained *In(2R)NS* (LINDSLEY and GRELL 1968). Inversions

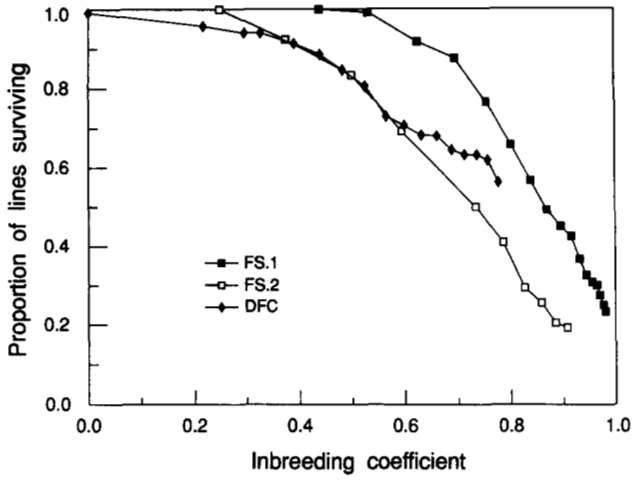

FIGURE 2.—Inbreeding depression. Proportion of lines surviving *vs.* inbreeding coefficient (F) in the full-sib 1 (FS.1), full-sib 2 (FS.2) and double first-cousin (DFC.1) treatments.

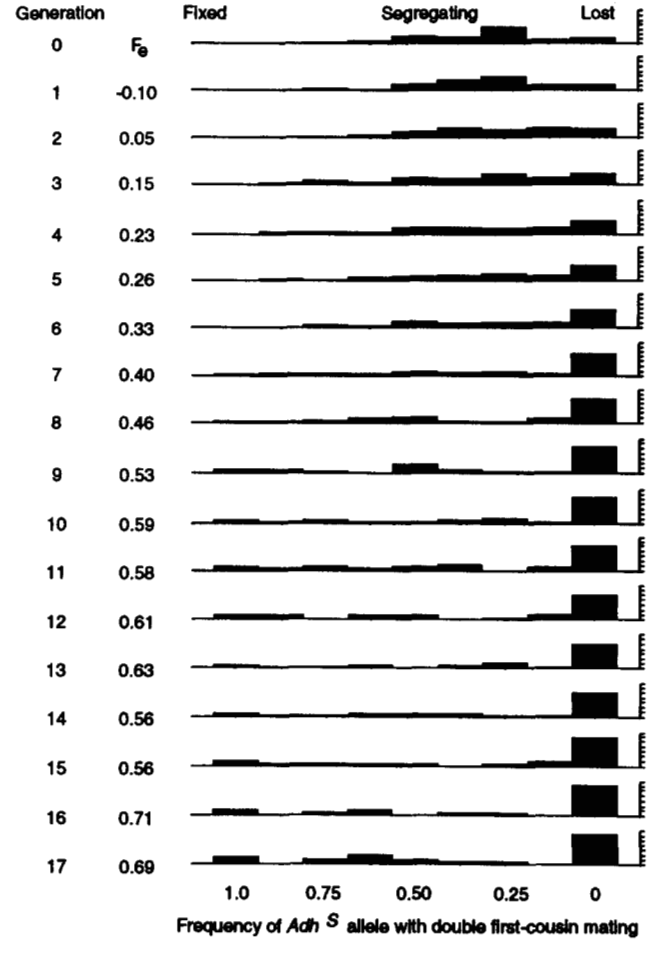

FIGURE 3.—Dispersion in the frequency of Adh^S over generations of inbreeding among surviving lines of double first-cousin 1 (DFC.1) treatment.

were absent from other regions of the genome. No inversions were found in a random sample of 30 larvae from a cage of population 2.

Lethals: Following unexpected results for the *Adh* locus in FS.2, two chromosome 2L lethals were identified and attempts made to map them. Homozygous lethality was found in four of seven Adh^S chromosomes and in two of seven Adh^F chromosomes sampled from 14 different flies from a FS.2 line that had been maintained by mass-mating approximately 50 flies for 60 generations following eleven generations of full-sib inbreeding.

To map the lethals (*1*), *1/Sp* vg^u *If* virgin females were mated to *1/Cy B1* L^4 test males and non-*Cy B1* L^4 progeny scored for *Sp*, vg^U *and If*. The Adh^S chromosome lethal mapped at 48.1, 1.9 map units from the *Adh* locus. The lethality in Adh^F chromosomes was not allelic to that of the Adh^S chromosomes. Two or more loci may have been involved, since the lethal mapping yielded ambiguous results.

Analyses: Inbreeding depression in the FS.1 treatments was assessed using a weighted regression of progeny numbers per pair on F.

Heterozygosities for each locus in each treatment at the final generation were compared with Hardy-Weinberg equilibrium expectations using chi-square tests. It was necessary to test in this way, rather than doing the usual test for agreement of genotype frequencies with Hardy-Weinberg expectations, as expectations for the rarest homozygote were less than 5 in many cases. A sign test for equality in number of cases above versus below expectations was carried out using a chi-square test. Heterozygosities were also compared with those expected under inbreeding, namely $2pq(1-F)$, or its three allele equivalent. Expected numbers of heterozygotes for this test were generally less than 5, so the comparison was done as a sign test using a chi-square test.

Observed and expected heterozygosities (genetic diversities) during the course of the experiment were followed by comparing the effective inbreeding coefficient F_e (see SING *et al.* 1973) with the expected pedigree inbreeding coefficient F. In the absence of disruptive forces such as selection, F_e has a theoretical expectation equal to WRIGHT's F. The effective coefficient of inbreeding in the tth generation $F_e(t)$ was estimated by

$$F_e(t) = \frac{H_0 - H_t}{H_0} \tag{1}$$

where H_0 is the expected frequency of heterozygotes in the parent generation, and H_t the heterozygosity at generation t. H_0 was calculated as

$$H_0 = 1 - \sum p_i^2 \tag{2}$$

where p_i is the frequency of the ith allele in the founder population. H_0, then, is the expected frequency of heterozygotes at zygote formation given the allele frequencies in the parents. Similarly H_t's in zygotes (prior to any selection), were predicted from $(t-1)$ mating types.

The standard error (SE) of F_e is

$$\text{SE}(F_e) = \frac{\text{SE}(H_t)}{H_0} \tag{3}$$

where $\text{SE}(H_t)$ is the standard error of heterozygosities at a locus in generation t.

TABLE 1

Genotype frequencies at the final assay in full-sib 1 (FS.1), full-sib 2 (FS.2) and double first-cousin (DFC.1) treatments, along with comparisons of heterozygosities with Hardy-Weinberg equilibrium expectations and $2pq(1 - F)$ expectations

Locus	N	Genotypes						$2pq(1 - F)$	F
		A_1A_1	A_1A_2	A_2A_2	A_1A_3	A_3A_3	A_2A_3		
FS.1									
α-*Gpdh*	60	0.767	(−)[a] 0.083**	0.150				0.007 (+)[b]	0.98
Mdh	56	0	0	1.000					0.98
Adh	56	0.571	(−) 0.214**	0.214				0.008 (+)	0.98
Odh	56	0.964	(+) 0.036	0.000				0.001 (+)	0.98
Aldox	56	0.054	(−) 0.036*	0.911				0.003 (+)	0.98
Est-6	56	0.804	(−) 0.089*	0.036	0.000	0.036	0.036	0.005 (+)	0.98
FS.2									
Mdh	30	0.233	(−) 0.167**	0.600				0.049 (+)	0.89
Adh	30	0.533	(+) 0.467	0.000				0.041 (+)	0.89
Idh	32	0.437	(−) 0.094**	0.469				0.070 (+)	0.86[c]
Odh	30	0.767	(+) 0.233	0.000				0.024 (+)	0.89
Mdh-N	30	0.667	(−) 0.167*	0.167				0.043 (+)	0.89
Est-6	30	0.267	(−) 0.067**	0.267	0.000	0.333	0.067	0.076 (+)	0.89
DFC.1									
α-*Gpdh*	88	0.716	(−) 0.125**	0.159				0.084 (+)	0.76[c]
Mdh	76	0	0	1.000					0.78
Adh	76	0.684	(−) 0.145**	0.171				0.082 (+)	0.78
Odh	76	0.934	(+) 0.066	0.000				0.014 (+)	0.78
Aldox	76	0.105	(−) 0.079**	0.816				0.055 (+)	0.78
Est-6	76	0.553	(−) 0.237**	0.211				0.098 (+)	0.78

* $P < 0.05$; ** $P < 0.01$.

[a] Direction of deviation of heterozygosity from Hardy-Weinberg equilibrium expectations: (−) represents a deficiency of heterozygotes.

[b] Direction of difference between observed and $2pq(1 - F)$ expectation for heterozygosity: (+) indicates an excess of heterozygotes.

[c] Data from the penultimate generation.

The mean effective coefficient of inbreeding ($\bar{F}_e$) was calculated from the average heterozygosity summed over all loci, as follows:

$$\bar{F}_e = 1 - \frac{\sum H_t}{\sum H_0} \tag{4}$$

with standard error

$$\text{SE}(\bar{F}_e) = \frac{\sqrt{\sum [H_0 \text{SE}(F_e)]^2}}{\sum H_0} \tag{5}$$

A direct test for natural selection was carried out by comparing observed and expected distributions of progeny genotypes from *segregating* mating types using chi-square tests. The expected frequencies of common allele homozygotes (CC), heterozygotes (CR) and rare allele homozygotes (RR) are

$$\begin{aligned} CC' &= (CC \times CR)/2 + (CR \times CR)/4 \\ CR' &= [(CC \times CR) + (RR \times CR) + (CR \times CR)]/2 \\ RR' &= (RR \times CR)/2 + (CR \times CR)/4 \end{aligned} \tag{6}$$

where the matings in the previous generations are shown in brackets. For CC (and RR) the chi-square value is half the squared sum of the χ_1 (signed) values for CC × CR (or RR × CR) and CR × CR (A. Robertson *personal communication*).

Data were tested for homogeneity using the binomial expectation calculated from observed genotype frequencies; the few segregations that were not homogeneous were omitted. Data from several generations were combined in these tests. The groupings are a compromise between the need to test for selection in lines with a wide range of different F values and a requirement for reasonable statistical power (dependent on allele frequencies and numbers of surviving lines).

Estimates of the magnitude of fitness differences among the genotypes were obtained using matrix analyses (see BARTLETT and HALDANE 1934; FISHER 1949). FISHER's (1949) mating type symbols are used in what follows (see APPENDIX, Table 6). The expected vector of mating type frequencies ($\mathbf{v}_t$) in the t th generation under the assumption of neutrality is

$$\mathbf{v}_t = \mathbf{A} \cdot \mathbf{v}_{t-1} \tag{7}$$

where $\mathbf{A}$ is the generation matrix and $\mathbf{v}_{t-1}$ is the observed distribution in the previous generation. Two parameters for the survival of the common and rare homozygotes (relative to heterozygote fitness of unity) were introduced as multipliers of elements and column divisors in the generation matrix, as shown for full-sib matings in the APPENDIX (Table 7). The generation matrix for DFC.1 is given by RUMBALL (1974). Maximum likelihood estimates of fitnesses were computed by equating the observed mating types in one generation with those of the previous generation premultiplied by the matrix. Although it was possible to estimate genotype fitnesses for most individual generations, more meaningful results were obtained by grouping generations into three or four phases of inbreeding. The fitnesses were estimated by simultaneously equating the observed and expected mating types of all pairs of consecutive generations in the group.

Two models of selection were assumed. In one, fitnesses were estimated solely *within* mating types; in the other, total fitnesses were estimated within and between mating types. Selection *within* mating types is due to the differential egg-adult survival of genotypes. Selection *between* mating types involves mainly fertility of lines (male fertility and mating ability, female

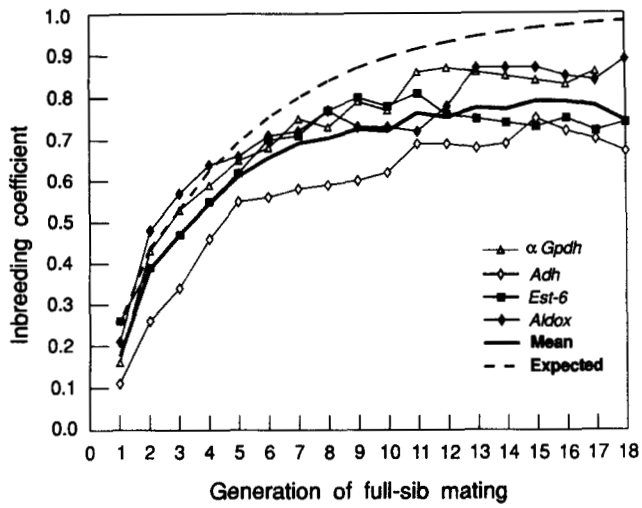

FIGURE 4.—Changes in expected and effective coefficients of inbreeding over generations for full-sib 1 (FS.1) loci.

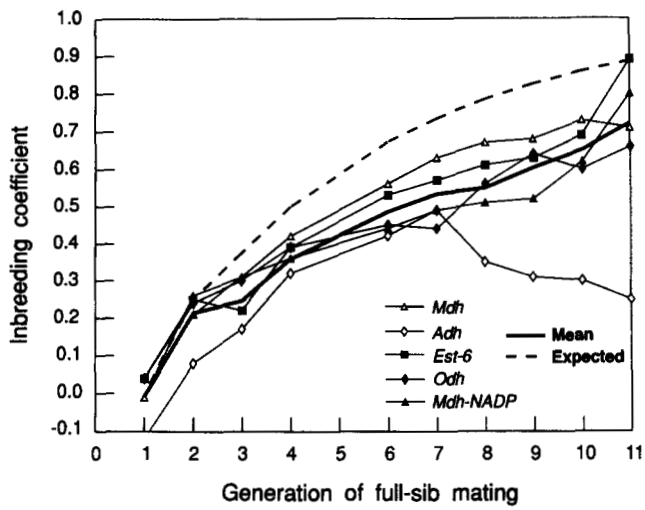

FIGURE 5.—Changes in expected and effective coefficients of inbreeding over generations for full-sib 1 (FS.2) loci.

mating ability and egg laying capacity) as a single fertile pair of progeny was sufficient to maintain a line.

RESULTS

Inbreeding depression was apparent from the substantial and significant loss of lines over time in all treatments (Figure 2). To make the measures in DFC comparable to those in the FS treatments, the square root of proportion of lines surviving in DFC was used as the equivalent of two FS lines had to both survive in DFC.1. The failure rate was 12.2% in 746 single pair matings from laboratory reared flies from a recent sample of the same Tyrrell's outbred stocks (M. E. MONTGOMERY, L. M. WOODWORTH, R. FRANKHAM and D. A. BRISCOE, unpublished data). Consequently, the probability of three pair matings all being unsuccessful (as required for our inbred lines to fail) is $0.122^3 = 0.0018$. The proportion of

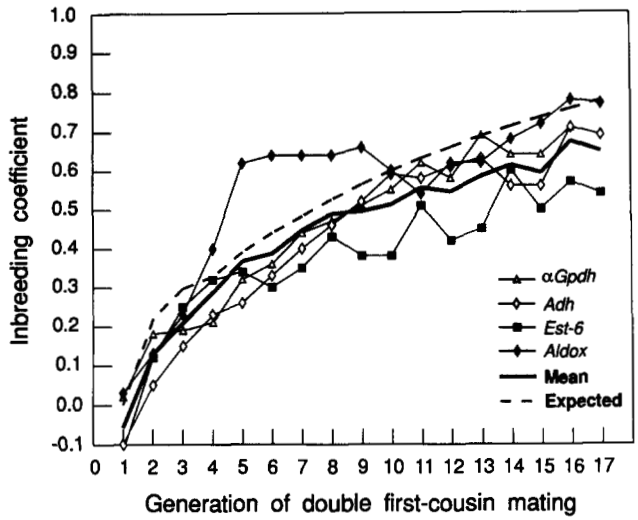

FIGURE 6.—Changes in expected and effective coefficients of inbreeding over generations for double first-cousin (DFC.1) loci.

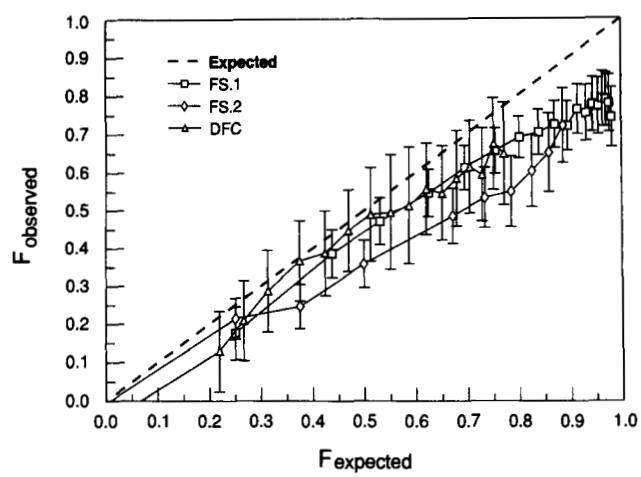

FIGURE 7.—Plots of mean effective inbreeding coefficients against expected inbreeding coefficients for the full-sib 1 (FS.1), full-sib 2 (FS.2) and double first-cousin treatments (DFC.1) treatments.

cumulative extinctions at the final generation that could be attributed to such base population level failures is 0.03 for FS.1 ($1 - [1 - 0.0018]^{18}$), 0.02 for FS.2 and 0.03 for DFC.1. The observed extinction levels of 0.77 ± 0.04, 0.81 ± 0.04 and 0.44 ± 0.07, respectively, greatly exceeded these values. The average number of progeny per mating in the FS.1 treatment declined significantly with inbreeding, the regression being −0.86 ±0.11 progeny per 1% increase in F ($P < 0.01$).

The distribution of allele frequencies across the lines diverged in the expected manner; this was most graphically illustrated by the dispersion of the *Adh* allele frequencies among DFC lines (Figure 3).

Heterozygosity was reduced in the inbred lines, as shown by the genotype frequencies at the final generations in each treatment (Table 1). Most loci (12/16)

TABLE 2

Test for natural selection in the full-sib 1 treatment (FS.1)

Locus	Generations (range of F)			
	1+2 (0.25–0.44)	3+4 (0.53–0.62)	5–9 (0.69–0.87)	10–17 (0.89–0.98)
αGpdh				
No. segregating lines	93	62	64	30
CC	0.57	1.35	(−) 12.68**	(−) 11.49**
CR	0.54	(+) 4.64*	(+) 28.12**	(+) 24.07**
RR	0.01	2.51	(−) 8.11**	3.79
Adh				
No. segregating lines	136	100	123	82
CC	(−) 11.69**	(−) 7.43**	(−) 7.97**	(−) 5.49*
CR	(+) 25.94**	(+) 9.68**	(+) 34.41**	(+) 26.56**
RR	(−) 8.93**	1.08	(−) 22.25**	(−) 16.40**
Est-6				
No. segregating lines	116	81	111	43
CC	3.64	0.11	1.63	(−) 6.65**
CR	(+) 14.50**	2.99	(+) 14.11**	(+) 31.44**
RR	(−) 8.77**	3.03	(−) 9.52**	(−) 13.08**

	Generations (range of F)	
	1–4 (0.25–0.62)	5–17 (0.69–0.98)
Odh		
No. segregating lines	15	36
CC	1.33	(−) 11.44**
CR	0.13	(+) 29.38**
RR	2.00	(−) 5.56*
Aldox		
No. segregating lines	50	71
CC	1.19	(−) 7.58**
CR	2.56	(+) 22.08**
RR	3.00	(−) 9.64**

Chi-square values for tests of deviation of common homozygote (CC), heterozygote (CR) and rare homozygote (RR) genotype frequencies from expected segregation ratios for segregating FS.1 mating types.

* $P < 0.05$; ** $P < 0.01$. (−) Deficiency of the genotype compared to the expectation. (+) Excess of the genotype compared to the expectation.

showed significant deficiencies of heterozygotes compared to Hardy-Weinberg equilibrium predictions, as expected. A notable exception was *Adh* for FS.2, which showed a non-significant excess of heterozygotes (see also Figure 5). A balanced lethal system on the second chromosome was responsible for this anomalous behavior.

There was an excess of heterozygotes compared to the $2pq(1 - F)$ expectation for all 16 informative locus-treatment combinations. The patterns of decline in heterozygosity are illustrated in Figures 4–6, showing the observed inbreeding (F_e) at each locus compared to expectation. The overall decline in heterozygosity was slower than expected at all FS.1 loci, at all FS.2 loci, except *Est-6* (for the last generation), and in DFC.1 at all loci except *Aldox*. Generally, the excess of heterozygosities over that expected increased with inbreeding.

The two comparable inbreeding treatments (FS.1 and DFC.1) behaved in a similar manner. There were no significant difference between FS.1, and DFC.1 in F_e at points of equivalent F (Figure 7). FS.2 values tended to have lower F_e's than FS.1 and DFC.1 until converging with them in later generations.

Heterozygotes showed higher egg-adult survival than homozygotes, as shown by the segregation ratios. This was evident at all loci in FS.1 (Table 2) and FS.2 (Table 3), except for *Mdh* in FS.2 (estimated over all generation of inbreeding). In DFC.1, the rare homozygotes were significantly less fit than the heterozygotes at each locus, but not significantly different from the common homozygote (Table 4).

Maximum likelihood estimates of the magnitude of reproductive fitness differences among genotypes within mating types, obtained from the matrix operations, are shown in Table 5 for all loci in FS.1 and DFC.1. All loci showed heterozygote advantage in the later generations (FS.1 generations 4–8 and DFC.1 generations 8–16) and all except α-*Gpdh* and *Aldox* for FS.1 generations 1–5 and *Adh* for DFC.1 generations 1–16. A similar picture of heterozygote advantage in viability was evident in FS.2 (results not shown). There were no significant differences in mean fitness between FS.1 and DFC.1 at comparable F levels. In general, as inbreeding increased the homozygotes appeared relatively less fit than heterozygotes in FS.1 (Figure 8). The estimates of

TABLE 3

Test for natural selection in the full-sib 2 (FS.2) treatment

Locus	Generations (range of F) 1+2 (0–0.25)	3–10 (0.37–0.89)
Mdh		
No. segregating lines	105	66
CC	(-) 7.15**	(-) 9.88**
CR	(+) 7.62**	(+) 7.76**
RR	0.18	0.25
Adh		
No. segregating lines	93	88
CC	1.23	(-) 8.35**
CR	(+) 15.68**	(+) 38.20**
RR	(-) 22.90**	(-) 15.33**
Odh		
No. segregating lines	94	61
CC	0.00	2.48
CR	3.06	(+) 20.49**
RR	(-) 5.65*	(-) 16.64**
	Generations (range of F) 1+2 (0–0.25)	6+7 (0.67–0.73)
Est-6		
No. segregating lines	110	29
CC	3.77	(-) 8.27**
CR	(+) 11.36**	(+) 9.93**
RR	(-) 6.12*	1.08
	Generations (range of F) 2+3 (0.25–0.37)	6+7 (0.67–0.73)
Mdh-NADP		
No. segregating lines	80	28
CC	0.11	3.00
CR	(+) 7.22**	(+) 8.64**
RR	(-) 9.92**	3.68

Chi-square values for tests of deviations of common homozygote (CC), heterozygote (CR) and rare homozygote (RR) genotype frequencies from expected segregation ratios for segregating FS.2 mating types.

* $P < 0.05$; ** $P < 0.01$. (-) Deficiency of the genotype compared to the expectation. (+) Excess of the genotype compared to the expectation.

total reproductive fitness (within and between mating types) showed a related picture of heterozygote advantage and lower fitness of rare than common homozygotes, though the magnitude of differences were generally less. The differences in magnitude of fitness differences probably result from the fact that differences were attributed solely to viability differences within matings in the former case (within fitness), but spread over viability differences, fertility differences and competition between reserve matings in the latter (total fitness) case.

In both FS systems, the mean frequency of the common allele (in surviving lines) increased during inbreeding, but there was no significant change in DFC.1 allele frequencies. The regression of the mean percentage change in common allele frequency per 1% increase in F_e was 0.095, 0.160 and 0.024 for FS.1, FS.2 and DFC.1, respectively. This increase in frequency of the common alleles was reflected in the fitness estimates; homozygotes for common alleles tended to be fitter than homozygotes for the rare allele. Over all generations, or at some stages, the common allele homozygote was fitter than the rare in FS.1: *Est-6*, FS.2: *Adh* and *Odh* and in DFC.1: *α-Gpdh*, *Adh* and *Aldox*, while the rare allele was fitter than the common in FS.2 *Mdh* (Tables 2–4).

DISCUSSION

Inbreeding resulted in all its expected consequences; fixation within inbred lines, genetic differentiation among lines and an overall decline in heterozygosity. Further, inbreeding depression was observed in all treatments. Such inbreeding depression has been documented many times since DARWIN (1876) described it (see WRIGHT 1977; CHARLESWORTH and CHARLESWORTH 1987; RALLS *et al.* 1988; FALCONER 1989). Consequently, the behavior of the inbred lines described herein was typical of inbred lines.

How rapidly did heterozygosity decline under rapid inbreeding? The decline in heterozygosity was significantly slower than predicted; about 80% of theoretical expectations for both full-sib and double first-cousin inbreeding. This is in accord with the body of (mainly circumstantial) evidence that the decline in heterozygosity under inbreeding is slower than predicted by theory (see Introduction). Of the studies published in detail, that by SING *et al.* (1973) with Drosophila enzyme markers is the nearest to this work. They followed allozyme frequencies in mass mated populations maintained using five inseminated females in a bottle per generation. Decline in heterozygosity and rate of fixation at most loci were faster than expected. However, when adjustments were made for a lower effective than census size (using the proportion of lines fixed), they inferred that there was a slower rate of decline in heterozygosity than expected from theory. Such problems of interpretation do not arise in pedigreed populations with single pair matings as used in this study. From preliminary data on inbreeding with enzyme markers, YEH and SCHEINBERG (1972) reported an excess of heterozygotes among inbred lines of *Tribolium castaneum*, and CHAPCO *et al.* (1973) reported persistent heterozygosity in a few full-sib lines of *D. melanogaster*, although over all lines heterozygosity at the *α-Gpdh* and *Adh* loci decreased approximately as expected. For the first time, the present study provides unequivocal evidence for slower than expected decline in heterozygosity under inbreeding in a substantial multi-generation study involving several loci.

The slower than predicted decline in heterozygosity can be attributed unambiguously to natural selection. In

TABLE 4

Test for natural selection in the double first-cousin (DFC.1) treatment

Locus	Generations (range of F) 1+2 (0–0.22)	3+4 (0.30–0.33)	5–9 (0.39–0.56)	10–17 (0.60–0.78)
αGpdh				
No. segregating lines	86	100	130	66
CC	3.06	3.07	0.00	(–) 5.00*
CR	(+) 6.72**	0.50	3.01	(+) 8.75**
RR	2.02	2.05	(–) 5.55*	3.30
Adh				
No. segregating lines	147	123	174	108
CC	1.37	3.42	(–) 4.92*	0.87
CR	1.97	(+) 7.87**	(+) 25.39**	(+) 8.16**
RR	(–) 10.56**	3.77	(–) 19.83**	(–) 7.98**
Est-6				
No. segregating lines	78	110	187	138
CC	0.00	1.24	2.36	(–) 6.65**
CR	0.32	0.65	(+) 12.36**	(+) 22.04**
RR	0.53	(–) 7.67**	(–) 8.59**	(–) 12.06**

	Generations (range of F) 1–4 (0–0.33)	5–16 (0.39–0.78)
Odh		
No. segregating lines	22	46
CC	0.22	1.22
CR	0.36	(+) 9.78**
RR	0.00	(–) 12.12**
Aldox		
No. segregating lines	28	58
CC		0.02
CR		1.69
RR		2.48

Chi-square values for tests of deviations of common homozygote (CC), heterozygote (CR) and rare homozygote (RR) genotype frequencies from expected segregation ratios for segregating DFC.1 mating types.

* $P < 0.05$; ** $P < 0.01$. (–) Deficiency of the genotype compared to the expectation. (+) Excess of the genotype compared to the expectation.

TABLE 5

Estimates of average fitnesses of homozygotes relative to a heterozygote fitness of unity in FS.1 and DFC.1 inbred lines

System	Generations	F	*αGpdh*	*Adh*	*Est-6*	*Aldox*
Mean fitness of homozygotes [(CC + RR)/2]						
FS.1	1–5	0.25–0.70	0.84	0.61	0.50	0.79
DFC.1	1–16	0–0.78	0.78	0.75	0.71	0.77
FS.1	4–8	0.62–0.84	0.48	0.50	0.41	0.41
DFC.1	8–16	0.53–0.78	0.61	0.67	0.48	0.64
Difference in fitness between common and rare homozygotes (CC – RR)						
DFC.1	1–16	0–0.78	+0.30	+0.79*	+0.05	+0.45*
FS.1	1–5	0.25–0.70	–0.32	–0.20	+0.23	+0.48
FS.1	1–17	0.25–0.98	–0.18	–0.01	+0.13	+0.14

* $P < 0.05$.

an outbred population, gene flow (contamination), mutation, and higher than expected effective population sizes can cause such deviations from expectations. However, all of these can be eliminated in the present study. Because both treatments employed single-pair matings there was no variation in family size and no gene flow. If mutations occurred, they could have been identified in all fixed and some segregating mating types and there was little chance of mistaken parenthood being undetected at all six loci.

Direct evidence for selection favoring heterozygotes was provided by the segregation ratios. As well, the fixed mating types had the highest extinction rates in both full-sib experiments, the rankings being t_R, t_C, u_R, v, u_C, for both FS.1 and FS.2. Some of these differences were significant (see RUMBALL 1974).

Selection favoring heterozygotes could operate through heterozygote advantage at the enzyme loci themselves or through associative overdominance built up during inbreeding. The former will be evident in the

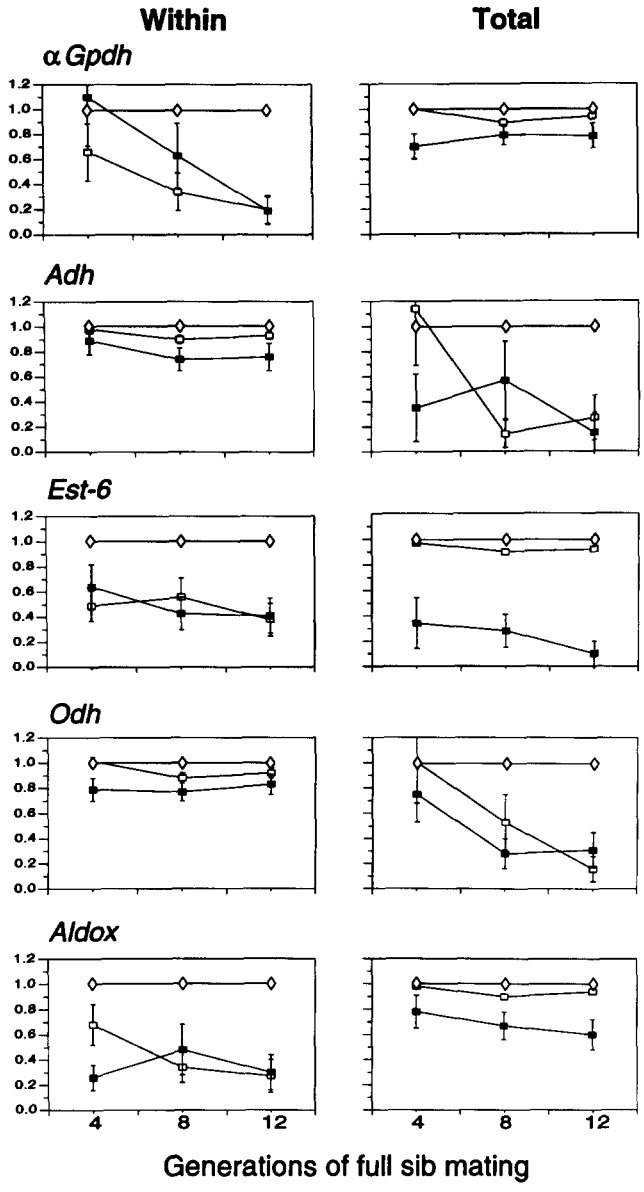

FIGURE 8.—Relative fitness of genotypes at five loci in FS.1 plotted against generations of full-sib mating for within mating types and for total fitness within and between types. The heterozygote is unity in all cases. The common homozygote is denoted by open symbols and the rare homozygote by closed symbols.

base population, while associative overdominance should only be evident in inbred lines. HAYMAN and MATHER (1953) showed that a 24% disadvantage of homozygotes compared to heterozygotes in survival within and between replaced lines is the minimum needed to avoid complete homozygosity in full-sib matings, whereas 15% suffices for double first-cousin matings. Such strong heterozygote advantage at individual enzyme loci is most unlikely in the light of evidence on the selective effects of allozymes in outbred populations (see LEWONTIN 1974; WRIGHT 1978; KIMURA 1983; HARTL and CLARK 1989). FRANKLIN (1981) surveyed Hunter Valley populations (including the one used in this study) over a five year period for the same allozyme loci used in this study and reported good agreement with Hardy-Weinberg equilibrium expectations, except for a small overall excess of heterozygotes at the *Adh* locus. The finding that heterozygotes were increasingly fitter than homozygotes over generations of inbreeding in nearly all loci is consistent with the expectations for associative overdominance. In the case of the *Adh* locus in FS.2, linked lethals were identified that resulted in associative overdominance. Balanced lethal systems probably arose in some FS.1 lines, but were not readily detectable. Because FS.2 had been established from a single mated female, the discrepancy around *Adh* was particularly noticeable. In a related study in chickens, MINA *et al.* (1991) presented evidence that associative overdominance was the most likely cause of excess heterozygosity. Consequently, it is most probable that the natural selection opposing fixation in the inbred lines is due, predominantly, to associative overdominance.

It was somewhat surprising that FS.1 and DFC.1 treatments showed similar heterozygote excess at similar F values and similar magnitudes of heterozygote advantage. Perhaps our observations are an artifact of the lower F values attained in DFC than FS.1. Alternatively, the magnitude of any differences may be too small to detect in an experiment of this size.

There have been no theoretical or simulation studies of the effects of full-sib or double first-cousin inbreeding in multilocus systems with many genes. The most relevant theoretical study is that by CHARLESWORTH (1991), who studied the effects of partial selfing in multilocus models with neutral and selected loci. All the models investigated, whether involving neutral and unlinked mutational load loci, neutral and mutation at completely linked loci, neutral and unlinked overdominant loci or neutral and linked overdominant loci, all led to apparent heterozygote advantage (associative overdominance) at the neutral loci. Consequently, our finding of apparent heterozygote advantage is to be expected. She predicted that the apparent fitness of the rare homozygote would be consistently lower than the common homozygote. This was a consistent feature of our experiments, and has also been found in plants (MITTON 1989). In general, the apparent fitness of homozygotes declined with increasing F. While this was not generally true in the models investigated by CHARLESWORTH (1991), neutral loci linked to overdominant loci showed this behavior. It is also likely that similar results may be obtained with linked deleterious mutations (see CHARLESWORTH 1991).

In conclusion, heterozygosity declined at a slower rate than predicted under both full-sib and double first-cousin inbreeding as a result of natural selection opposing fixation.

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APPENDIX

Fisher's mating type designations are given in Table 6 and the generation matrix is shown in Table 7.

TABLE 6

Fisher's full sib (FS) mating type designations

t-like homozygotes (t_c is CC × CC and t_R is RR × RR),
u one homozygote and heterozygote (u_c is CC × CR, u_R is RR × CR),
v two heterozygotes (CR × CR),
w unlike homozygotes (RR × CC),
x two heterozygotes with one like allele (01 × 02),
y one homozygote and a heterozygote of two different alleles (00 × 12) and
z two unlike heterozygotes (01 × 23).

TABLE 7

Generation matrix for full-sib matings with two alleles per locus, with parameters x and y for the survival of the common and rare homozygotes

	t_c	t_R	u_c	u_R	v	w
t_c'	x		x^2		x^2	
t_R'		y		y^2	y^2	
u_C'			$2x$		$4x$	
u_R'				$2y$	$4y$	
v'			1	1	4	1
w'					$2xy$	
Lost	$(1 - x)$	$(1 - y)$	$(1 + x)(1 - x)$	$(1 + y)(1 - y)$	$(2 + x + y)(2 - x - y)$	
W	x	y	$(1 + x)^2$	$(1 + y)^2$	$(2 + x + y)^2$	1
$W + B$	1	1	$2(1 + x)$	$2(1 + y)$	$4(2 + x + y)$	1