

INBREEDING: ITS EFFECT ON RESPONSE TO SELECTION FOR PUPAL WEIGHT AND THE HERITABLE VARIANCE IN FITNESS IN THE FLOUR BEETLE, *TRIBOLIUM CASTANEUM*

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Abstract.—We report our studies of the effect of inbreeding on the response to selection for increased pupal weight in the flour beetle, *Tribolium castaneum*. We also report the effects of inbreeding and selection for pupal weight on the heritable variation in fitness and fitness components. We created replicate and independent inbred lines with F -values of 0.00, 0.375, and 0.672, by 0, 2, and 5 generations, respectively, of brother-sister mating of adult beetles from an outbred stock population. Subsequently, we imposed artificial within-family selection for increased pupal weight in each of 15 inbred lines for eight generations; each line had its own paired, unselected control. We compared the response to selection across the three levels of inbreeding with theoretical expectation, and investigated the effects of inbreeding and selection on fitness variation among families within all 30 selected and control lines. Among-line variation in pupal weight increased with increased inbreeding prior to selection but diminished with directional selection. Inbreeding reduced the realized heritability of pupal weight concordant with quantitative predictions of additive theory. Mean fitness, measured in several ways, declined with inbreeding and declined further with selection. In contrast, the genetic variation for fitness in the inbred and selected lines equalled or exceeded that of the outbred controls. Our results suggest that inbreeding and selection may affect traits in different ways depending on the relative amounts of additive and nonadditive genetic variation.

Key words.—Additive genetic variance, fitness effects, inbreeding, nonadditive genetic variance, realized heritability, selection.

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Natural populations of many organisms experience “bottlenecks,” periodic reductions in the numbers of breeding adults (Powell 1978; Carson and Templeton 1984; McCauley 1989; Whitlock 1992a). “Founder events” in the colonization of island, marginal, or novel habitats can result in a temporary reduction in the numbers of breeding adults (e.g., Mayr 1942; Carson 1975; Powell 1978; Templeton 1980; Carson and Templeton 1984; Dodd and Powell 1985; Wade and McCauley 1988; Dodd 1989; McCauley 1989; Whitlock and McCauley 1990; Whitlock 1992a). Bottlenecks or founder events enhance genetic drift (Wright 1921, 1952; Wade and McCauley 1988; Whitlock and McCauley 1990; Whitlock 1992a,b). Subsequently, reduced population size at one time can lead to matings between close relatives (inbreeding) in later generations and further increase homozygosity (Nei et al. 1975). In addition, strong directional selection at one locus is experienced as a reduction in effective population size by the rest of the genome, especially in closely linked regions (Crow and Morton 1955). Thus, bottlenecks, founder events, and strong directional selection, alone or in combination, may limit the evolutionary potential of a population by causing a decrease in genetic variation inversely proportional to the effective population size (Wright 1952; Crow and Kimura 1970; Hartl and Clark 1989).

Components of genetic variance, such as dominance variance or epistatic variance, also change with inbreeding, bottlenecks, founder events, or strong directional selection on loci of major effect (Robertson 1952; Charlesworth and Charlesworth 1987; Goodnight 1987, 1988; Cockerham and Tachida 1988; Tachida and Cockerham 1989; Whitlock et al. 1993). Changes in these components of variance affect the

amount of additive genetic variance remaining within a local population for adaptation after a reduction in population size. Theoretical studies of inbreeding with dominance variance (Robertson 1952; Whitlock et al. 1993; Willis and Orr 1993) have shown large relative increases in the additive genetic variance and smaller absolute increases in heritability as a result of inbreeding. Goodnight (1987, 1988) discovered a similar effect of additive-by-additive epistasis (i.e., gene interactions between loci; Wade 1992a). Founder events or other reductions in effective population size “convert” nonadditive genetic variation to additive (i.e., heritable) genetic variation on a local scale (Goodnight 1987, 1988; Wade, 1992b; Whitlock et al. 1993; Whitlock 1995). By “convert,” we mean that gene interactions in an ideal building-block model (sensu Cockerham) contribute to estimated allelic effects when the frequencies of interacting genes change stochastically in finite populations (cf. Wade 1992b, p. 55–56). Thus, when there are interactions among loci, a population bottleneck may sometimes enhance rather than diminish the possibility for adaptive evolution by increasing the additive genetic variance for fitness. This effect depends critically upon the nature of the additive and nonadditive genetic variance within the outbred source population from which the founders or colonists derive (Goodnight 1987, 1988; Wade 1992b; Whitlock et al. 1993; Whitlock 1995). For example, an increase in additive variance for fitness resulting from an increased homozygosity of deleterious recessive genes is unlikely to lead to evolutionary novelty whereas, a similar increase, resulting from epistatic gene action, might be of evolutionary and adaptive importance.

We studied the effects of inbreeding by brother-sister mat-

ing on the subsequent response to artificial directional selection on pupal weight and the indirect effects of inbreeding and selection on fitness components. We imposed inbreeding prior to selection on an outbred base population of the flour beetle, *Tribolium castaneum*. Using three different, replicated levels of inbreeding, we selected within families for increased pupal weight for eight generations. Each of the 15 selected lines had a paired, unselected control (30 lines total). We measured the direct effects of this selection on the mean and heritable variance of pupal weight, an additively determined trait (Enfield 1980). We also measured the indirect effects of this selection and inbreeding on total fitness, offspring numbers, and longevity. Total fitness has a significant nonadditive component in flour beetles (Wade 1985; Wade and Goodnight 1991). Because mean fitness often declines with inbreeding and artificial selection, we investigated changes in the mean as well as the heritability of fitness with selection.

In this paper, we report our results for the mean and variance of the direct response of pupal weight and the indirect responses of fitness and fitness components to selection as a function of the level of inbreeding. We compare our results with expectations from additive theory. In the discussion, we review similar experimental studies that have investigated inbreeding and selection simultaneously or inbreeding alone, without selection, in light of our findings and discuss the implications for evolution in subdivided populations.

MATERIALS AND METHODS

We established three inbreeding treatments, corresponding to 5, 2 and 0 generations, respectively, of brother-sister mating from a large outbred laboratory population of *T. castaneum* (c-SM) maintained at the University of Chicago (cf. Wade [1977, 1985] for details concerning this stock). The expected inbreeding coefficients for the three treatments are $F_5 = 0.672$, $F_2 = 0.375$, and $F_0 = 0.00$, where the subscripts indicate number of generations of brother-sister mating. We independently derived five replicates for each of these three inbreeding levels from the c-SM stock. After inbreeding, we derived one control and one selected line from each replicate to establish 15 pairs of control and experimental lineages. We maintained each control and experimental line as 20 randomly mating, single pairs of adults with an equal contribution of each pair (one male and one female) to the next generation. It is important to emphasize that, before selection, the same 20 families were common to each control-experimental pair.

At each generation in each selected line, we removed five male and five female pupae from each family (3 inbreeding levels \times 5 replicates/inbreeding level \times 20 families/replicate \times 2 sexes/family \times 5 pupae/sex = 3000 pupae total at each generation) and weighed them to the nearest 0.0001 g using an analytical balance. We placed the heaviest male and female pupae from each family in separate shell vials containing 4 g of flour medium (95% sifted whole wheat flour, 5% brewers' yeast). After the pupae eclosed, we paired each male adult randomly with a single female using a table of random numbers. We emptied the contents of the male's vial into the vial of his mate so that the total volume of medium available for the progeny of each pair was 8 g. A similar procedure was

used in the control lines but one male and one female pupae were removed from each family, weighed, and randomly paired (3 inbreeding levels \times 5 replicates/inbreeding level \times 20 families/replicate \times 2 sexes/family \times 1 pupa/sex = 600 pupae total per generation). This protocol maintained the initially established inbreeding levels and the 20 selected and 20 control families for each of the 15 inbreeding replicates because an equal number of offspring per family was used to establish each generation. The variance effective population number for each control lineage was in the range of 70–80 breeding adults based upon previous studies of the effective population size in this species and strain of flour beetles (Wade 1980, 1984; Wade and Goodnight 1991). Little additional genetic differentiation among control lineages would be expected to develop during the eight generations of the experiment ($< 5\%$).

All culture vials were maintained in incubators at 29°C with 70% relative humidity. In the analysis of the response to selection, we used the control populations as a covariate to adjust for uncontrolled environmental trends during the experiment because this permits "relatively unbiased estimates of response" (Muir 1986). Because our controls and experimental replicates are closely paired after inbreeding but before selection, they permit us a better estimate of genetic parameters which can vary among lineages (Hill 1977).

We estimated $h^2(0)$, the heritability of pupal weight in the outbred stock prior to inbreeding and selection. We used a balanced half-sib design with 50 randomly chosen sires, each mated to three randomly chosen females, and weighed five male and five female offspring from each of the 150 families (50 sires \times 3 dams/sire \times 10 offspring/dam = 1500 individuals). The heritability of pupal weight in the c-SM stock prior to selection and inbreeding was estimated as $h^2(0) = 0.431 \pm 0.081$. (Sire and dam estimates were combined because the sire estimate alone exceeded that of the dams [cf. Becker 1986, p. 59 for practical formulas.]) This estimate can be improved by combining it with the independent estimates of realized heritability from the five noninbred F_0 selection lineages (see Results below).

To examine the effect of inbreeding and selection on an additive character, pupal weight, at each generation, we calculated realized heritabilities and heritabilities estimated by full-sib analysis and parent-offspring regression. For each replicate, the heritabilities estimated from the covariance among full-sibs and from the parent-offspring regression were pooled in the manner recommended by Hill and Nicholas (1974, p. 452, eqs. [8–10]) to achieve a minimum variance estimate.

We compared observed heritabilities with the theoretically expected heritabilities using the formula from Falconer (1989, p. 247) for the change in heritability of an additively determined character with inbreeding: $h^2(F) = [h^2(0)] [1 - F]/[1 - Fh^2(0)]$. Using this formula, the expected heritabilities for the three experimental levels of inbreeding were $h^2(5) = 0.157$, $h^2(2) = 0.266$, and $h^2(0) = 0.367$. The latter estimate is a weighted estimate combining heritability data from the outbred stock, c-SM, and the outbred F_0 lineages (see below).

The Direct Effects of Inbreeding and the Indirect Effects of Selection on Fitness Components

We examined the effect of inbreeding and selection for increased pupal weight on the mean and heritability of several fitness components: (1) production of adult offspring by an individual in a test population (AO); (2) total offspring produced by a single pair of adults reared alone (TO); and, (3) longevity measured as the fraction of a cohort of 20 adults surviving to 180 d old (L). The first fitness component, AO, is probably closest to a measure of total fitness because it includes individual mating success, fertility and/or fecundity, hatching, and larval survivorship within a population as defined below. The second fitness component, TO, is the total number of offspring (larvae, pupae, and adults) produced by a single pair of adults in 8 g of medium. This is different from AO because it is not measured in the context of the population (cf. Wade 1979; McCauley and Wade 1980). These measures of fitness components were taken for individuals from each family in each replicate at different generations during the experiment.

We first measured the effects of inbreeding on the mean and variance of AO, the numbers of adult offspring produced by a single individual within a population in a 56-d period (Wade 1985). After the inbreeding and before selection, we measured AO by collecting three male and three female offspring from each replicate of each inbreeding treatment (3 inbreeding levels \times 5 replicates/level \times 2 sexes/replicate \times 3 individuals/sex = 90 individuals). Each of these "target" individuals was placed in an 8 dram vial with 8 g of medium with seven adults of the same sex and eight adults of the opposite sex; the latter 15 adults were all homozygous for a semidominant black body color mutation segregating in the c-SM stock (cf. Wade 1980, 1984, 1985; Wade and Goodnight 1991). We removed the 16 adults after two weeks and censused the adult offspring in each culture six weeks later and scored them for genotype. The offspring of the target individual were clearly identifiable by body coloration as +/b heterozygotes. The proportion of heterozygous (+/b) offspring of the each of the 90 target individuals was arcsine square-root transformed. Using these data, we estimated the effect of inbreeding on the mean and the variance of AO prior to selection.

We measured TO during the artificial selection experiment in generations 4, 5 and 6, as pair productivity in 45 days of culture. Heritabilities were estimated by parent-offspring regression across generations 4 and 5 and generations 5 and 6.

The longevity component of fitness, L, was measured after the eighth generation of selection by maintaining single sex cultures of 20 males or 20 females from all lines for 180 days. We scored longevity as proportion surviving to 180 days, approximately the median length of adult life for the more productive strains of *T. castaneum* (Mertz, Park, and Youden 1965).

RESULTS

The Effects of Inbreeding and Selection on Pupal Weight

Response to selection for increased pupal weight.—The mean pupal weight of males and females for each experi-

mental lineage and its control are graphed in Figure 1. For the majority of selected lineages, there is a clear tendency for pupal weight in the selected line to exceed that of its unselected control in the later generations. To test the statistical significance of the response to selection, we first regressed the mean weight of an experimental treatment on the mean weight of its control and then regressed the residuals on time measured in generations. The change in this difference between the experimentals and the controls over generations is a powerful statistical test for a response to selection in the presence of the between-generation environmental variation evident in Figure 1 (Muir 1986).

The response to selection measured as the slope of the residual with time was positive in 29 of the 30 cases (Table 1; sign test, $P < 0.005$), indicating an increasing difference in mean pupal weight between experimentals and controls as would be expected with successful directional selection. Thus, artificial selection for increased pupal weight produced a positive response at all levels of inbreeding and in essentially all replicates. To increase our statistical power for testing the effects of inbreeding on selection, we pooled the residual data across lineages within levels of inbreeding. We find that the difference in pupal weight between the experimentals and controls significantly increases with time for both sexes for all levels of inbreeding (Table 2). Both sexes responded to selection in all three inbreeding treatments.

Theoretical considerations (see above) and inspection of the slopes in Table 1 suggest that the rate of response to selection declines as inbreeding increases. We tested the significance of the variation in response to selection with inbreeding level by nonparametric comparison of the slopes of the five independent lineages for each inbreeding level. The slope (rate of increase with time) is highest for the outbred, F_0 , lineages, intermediate for the F_2 lineages, and lowest for the most inbred, F_5 lineages (Jonkheere's Test of Ordered Alternatives, $P < 0.005$ for both sexes [Hollander and Wolfe 1973, p. 139]). We conclude that the response to selection for increased pupal weight diminishes with increased prior history of inbreeding as expected from theory.

Although mean pupal weight was similar among treatments at the start of the experiment, the differences in response to selection among treatments resulted in differences among treatments in average pupal weight in the later generations (Table 3). In generations 5 through 8, the magnitude of the difference between the experimental and control mean pupal weights follows the order of inbreeding: $F_0 > F_2 > F_5$ (Jonkheere's Test of Ordered Alternatives, P values given in Table 3).

In summary, the magnitude of the response to selection varied with the prior history of inbreeding as predicted by additive theory: the largest response was associated with the outbred lineages (F_0), the smallest response with the most strongly inbred lineages (F_5), and an intermediate response with the intermediate level of inbreeding (F_2). There were no statistically significant trends, tendencies, or patterns among inbreeding levels in the variability of response to selection: within a level of inbreeding, all five replicates responded homogeneously (although our power to detect heterogeneity is not great).

Realized Heritability of Pupal Weight.—In the analysis

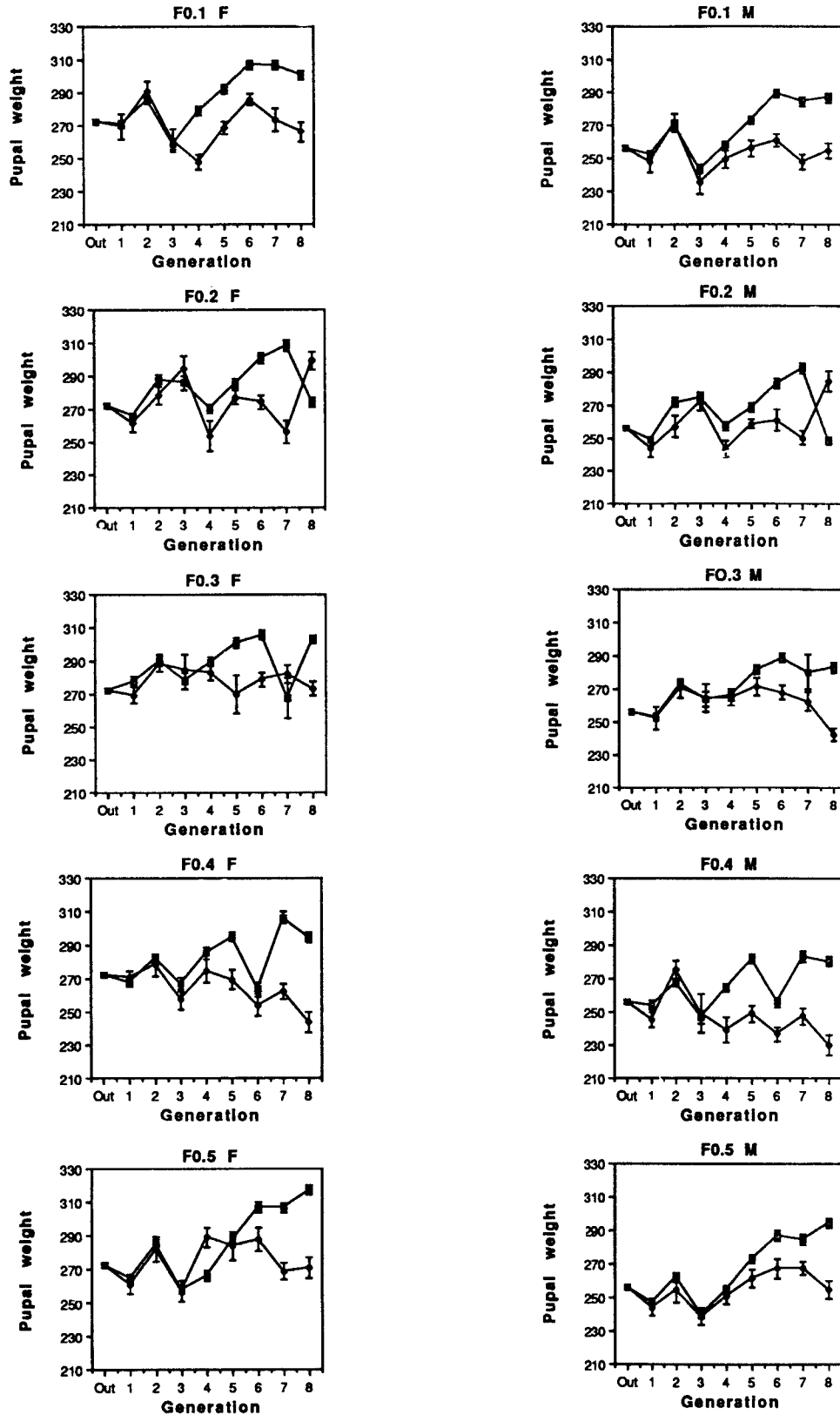


FIG 1. The mean and one standard error of pupal weight for males (M) and females (F) for each generation in each of the 15 experimental lineages (open squares) and the 15 paired control lineages (solid circles). The symbols, F0.i, F2.i, and F5.i, indicate the level of inbreeding prior to selection and the values of *i* (=1, 2, . . . , 5) indicate replicate. Thus, F0.1, is a graph of the mean pupal weight of females in replicate one of the experimental selection treatment with no inbreeding and its paired, unselected control.

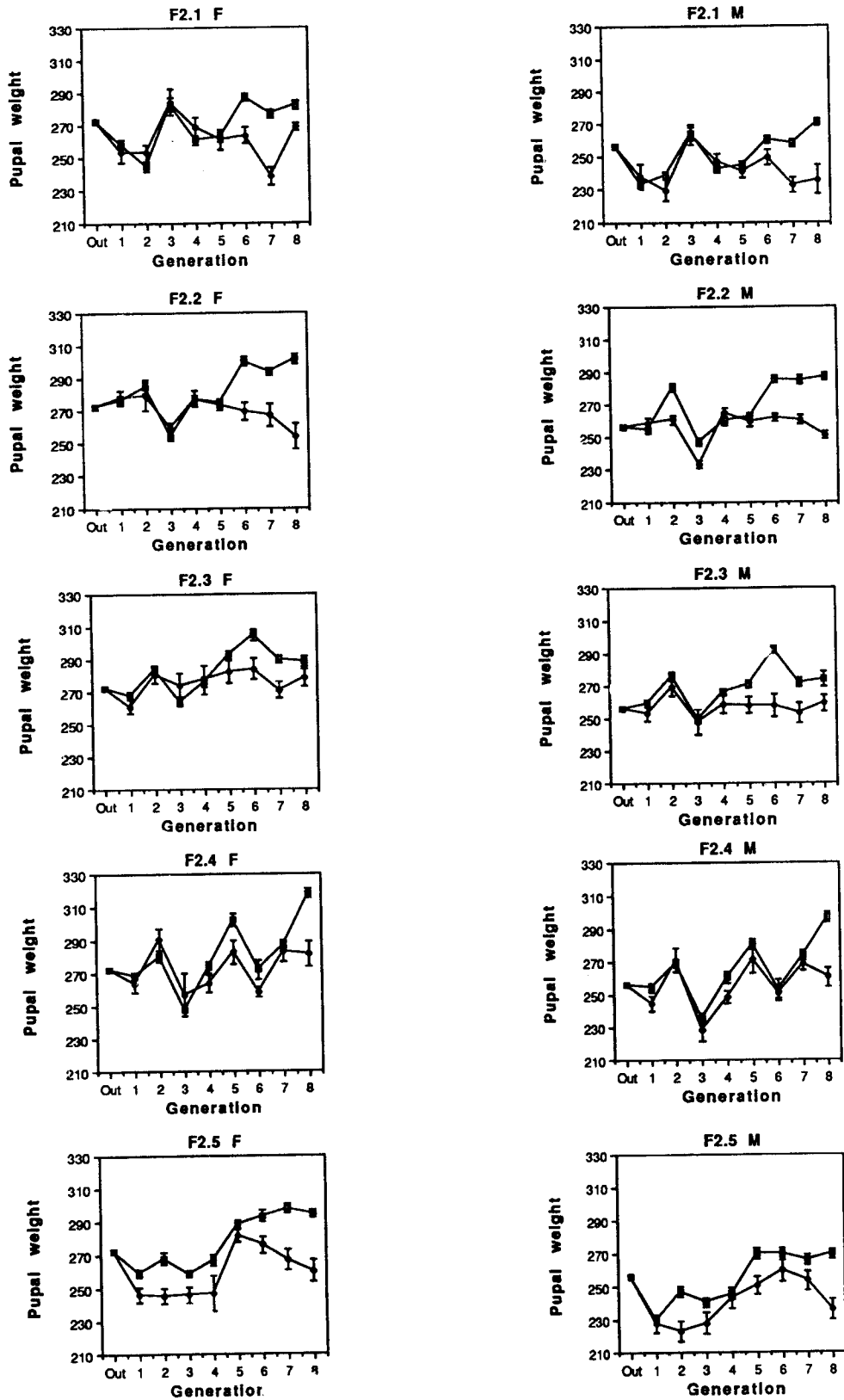


FIG 1. Continued.

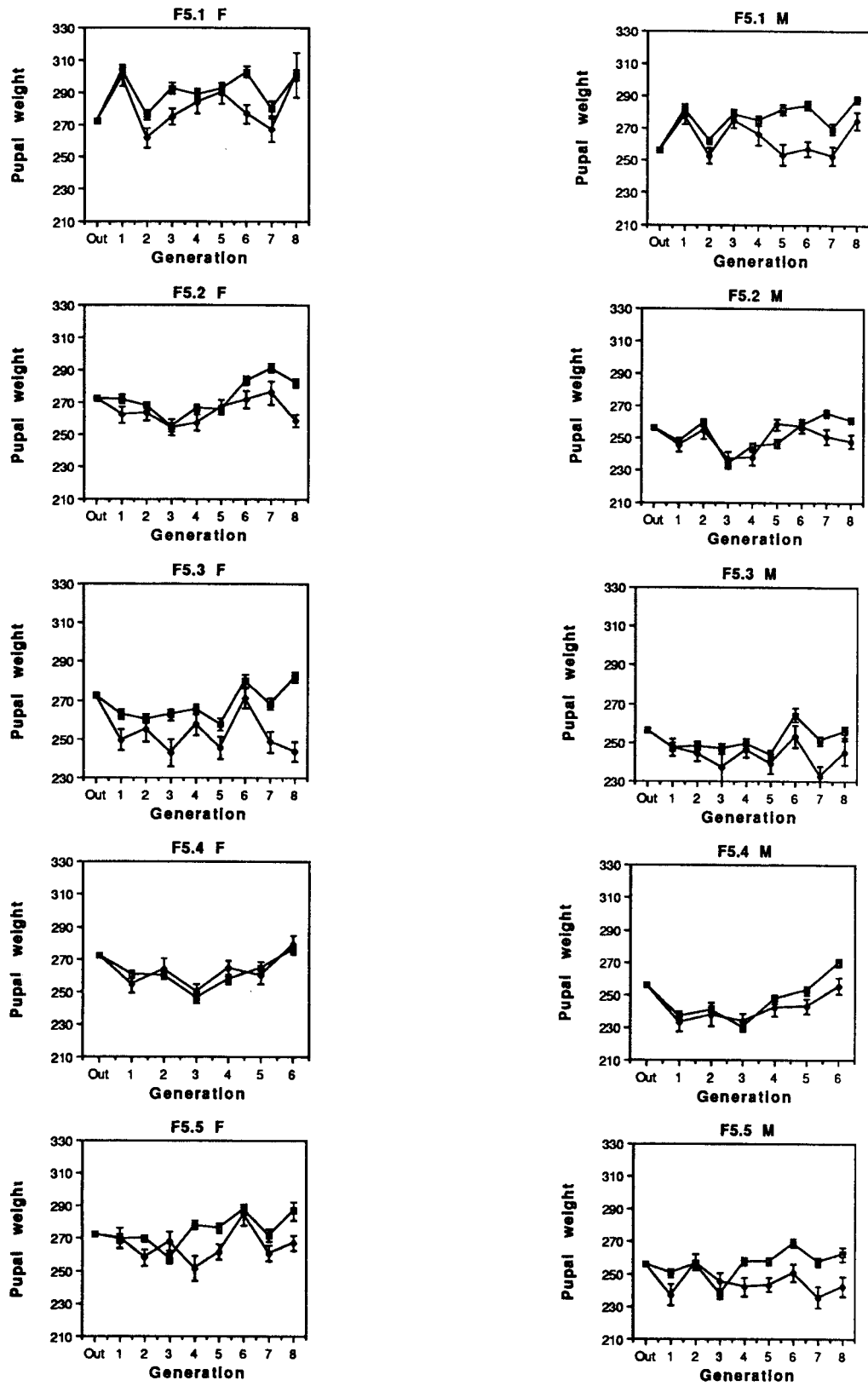


FIG 1. Continued.

TABLE 1. Slope and standard error (SE) of regression of residuals for experimental on control mean pupal weight on generation number for males (M) and females (F) in each lineage within the three levels of inbreeding F₀, F₂, and F₅.

Inbreeding Lineage		Females		Males	
		Slope	SE	Slope	SE
F ₅	1	0.084	1.043	1.491	0.998
	2	1.863	1.132	1.606	1.222
	3	2.623	0.946	1.619	0.570
	4	0.025	1.408	-0.186	0.071
	5	2.391	1.227	2.295	1.165
F ₂	1	4.276	1.582	4.391	1.124
	2	3.728	2.123	4.117	1.713
	3	2.158	1.448	2.721	1.325
	4	4.224	1.865	2.521	1.622
	5	2.508	1.158	2.951	1.446
F ₀	1	5.985	1.051	5.513	0.773
	2	5.663	1.778	4.848	1.505
	3	1.683	2.064	4.240	0.965
	4	3.730	2.093	3.460	1.845
	5	7.084	2.047	2.700	1.481

above, we examined the absolute response to selection in the experimental treatments relative to the paired controls. The treatment differences in absolute response could be owing to differences in either the selection differentials or the heritabilities. Thus, the absolute response to selection described above can be less informative than the response relative to the imposed selection differential (Falconer 1989). The standardized selection differential is the number of standard deviations by which the selected parents exceed the mean before selection. It is calculated as the mean of the selected parents minus the mean of all measured beetles before the parents are selected (i.e., including the selected parents) and divided by the standard deviation before selection (Lande and Arnold 1983; Falconer 1989). The lineage-means of the imposed selection differentials for each inbreeding level and each sex are given in Table 4 along with the empirical standard errors. The imposed artificial selection differentials appear homogeneous across treatments since there are no significant differences among the inbreeding treatments in average selection differential. Furthermore, the total cumulative selection differentials, averaged across males and females, were within 1.4% of one another: 9.993 for F₅, 10.004 for F₂, and 10.134 for F₀.

We estimated the realized heritability (Table 5) for each sex and lineage separately as twice the regression of the standardized response to selection on the cumulative selection differential. (The realized heritability is twice the re-

TABLE 2. Regression of pooled residuals for experimental on control mean pupal weight on generation for inbreeding levels F₀, F₂, and F₅.

Inbreeding	Sex	Slope	SE	F	P <
F ₅	F	1.539	0.478	10.365	0.003
	M	1.528	0.414	13.641	0.001
F ₂	F	3.379	0.678	24.803	0.0001
	M	3.340	0.594	31.657	0.0001
F ₀	F	4.727	0.806	34.365	0.0001
	M	4.072	0.566	51.872	0.0001

TABLE 3. The results by sex and generation of Jonkhere's Test of Ordered Alternatives to investigate whether the difference between experimental and control mean pupal weights occurs in the theoretically expected order, namely, F₅ < F₂ < F₀. The test statistic is J and P is the level of probability associated with J.

Generation	Females		Males	
	J	P <	J	P <
1	26	0.500	41	0.250
2	27	0.500	35	0.500
3	33	0.500	35	0.500
4	35	0.500	39	0.400
5	54	0.050	47	0.200
6	54	0.050	49	0.150
7	49	0.005	46	0.010
8	39	0.100	51	0.005

gression because selection was within families [Falconer 1989, p. 267].) In 28 of the 30 cases (Table 5), the heritability estimates were positive (sign test, P < 0.005). Both negative estimates fall in the most highly inbred, F₅ treatments and are very close to zero. As a group, realized heritabilities in the most inbred F₅ lineages were lower than in the F₂ lineages which were in turn lower than those in the outbred F₀ lineages (Jonkheere's Test of Ordered Alternatives, F₅ < F₂ < F₀ with P < 0.005 in males and P < 0.025 in females). With few exceptions, the theoretically expected value is close to the observed median value of realized heritability for all levels of inbreeding (Table 5; see also discussion in section Combined Heritability Estimates below).

Heritabilities Estimated from Parent-offspring Regression and Intra-class Correlation of Sibs.—The experimental design permits us to estimate heritabilities in each of the 15 selected lineages from parent-offspring regression across generations and from the intra-class correlation of sibs within generations. These separate estimates can be combined to obtain a minimum variance estimate using the iterative method recommended by Hill and Nicholas (1974, p. 452). Minimum variance estimates are especially useful in nonpara-

TABLE 4. Average selection differential within lineage by sex and generation for each of the three levels of inbreeding F₀, F₂, and F₅.

Gen.	Sex	F ₅		F ₂		F ₀	
		Mean	SE	Mean	SE	Mean	SE
1	F	1.348	0.022	1.399	0.123	1.404	0.014
	M	1.351	0.027	1.411	0.015	1.371	0.039
2	F	1.379	0.034	1.356	0.032	1.356	0.051
	M	1.341	0.040	1.301	0.051	1.225	0.021
3	F	1.292	0.037	1.273	0.025	1.313	0.029
	M	1.293	0.011	1.353	0.031	1.377	0.038
4	F	1.339	0.030	1.392	0.046	1.358	0.039
	M	1.360	0.027	1.317	0.045	1.376	0.034
5	F	1.255	0.044	1.358	0.045	1.341	0.025
	M	1.266	0.077	1.356	0.048	1.355	0.018
6	F	1.356	0.023	1.217	0.053	1.394	0.024
	M	1.296	0.026	1.296	0.036	1.282	0.034
7	F	1.406	0.019	1.296	0.015	1.315	0.036
	M	1.395	0.010	1.346	0.027	1.378	0.041
8	F	1.312	0.033	1.338	0.023	1.424	0.022
	M	1.312	0.052	1.322	0.020	1.392	0.022
Total		9.993		10.004		10.134	

TABLE 5. Realized heritabilities for pupal weight for the five selected lines within each inbreeding level, F_0 , F_2 , and F_5 , calculated as twice the regression of the total response to selection (R) on the cumulative selection differential (S_{cum} ; see also Figure 2).

Line	Sex	F_0	F_2	F_5
		h^2	h^2	h^2
1	F	-0.023	0.286	0.380
	M	0.141	0.258	0.441
2	F	0.181	0.344	0.068
	M	0.096	0.402	0.159
3	F	0.201	0.122	0.224
	M	0.090	0.175	0.517
4	F	-0.048	0.240	0.468
	M	0.182	0.144	0.420
5	F	0.146	0.073	0.369
	M	0.161	0.118	0.312
Median	F	0.146	0.240	0.369
Median	M	0.141	0.175	0.420
Expected		0.157	0.266	0.367

The magnitude of female and male heritabilities is $F_5 < F_2 < F_0$ (Jonkhere's test, $P < 0.025$ and $P < 0.04$, respectively).

metric statistical comparisons because they diminish the likelihood of errors in rank ordering of estimates by magnitude. The minimum variance estimates for each level of inbreeding at each generation are presented in Table 6. Treating each generation as a block with three estimates, we find, as in the above section, the pooled minimum variance estimates of heritability to be in the theoretically predicted order, $F_5 < F_2 < F_0$ (Page's test of ordered alternatives, $L = 96.5$, $P < 0.001$, Hollander and Wolfe [1973], p. 139).

Combined Heritability Estimates.—The best estimate of heritability in the outbred base population is obtained by combining three separate estimates: (1) the half-sib correlation of the outbred stock, c-SM, before inbreeding and selection (0.431, SE = 0.084); (2) the mean realized heritability estimate from the five independent outbred (F_0) selection lineages (0.335, SE = 0.058); and, (3) the mean of the pooled minimum variance estimates from parent-offspring regression, also from the five independent F_0 selection lineages (0.308, SE = 0.035). These three estimates were weighted by the inverse of the variance and averaged to obtain an estimate for the outbred stock of $h^2(0) = 0.367$. Using formula 15.1 (Falconer 1989, p. 247), we calculate the expected heritability for the F_5 and F_2 treatments as $h^2(5) = 0.157$ and $h^2(2) = 0.266$. Combining the estimates of realized heritability and the pooled minimum variance estimates for the F_5 and F_2 treatments, again weighted by the respective variances, we find that the observed heritabilities were $h^2(5) = 0.129$ and $h^2(2) = 0.223$, within 15% of theoretical expectation (cf. Table 6, last row).

Among-lineage Variance in Mean Pupal Weight before Selection.—The variance in mean pupal weight among-lineages within level of inbreeding was estimated at generation 1 before selection was imposed. The among-lineage variance in mean pupal weight as a fraction of the total variance was greatest, 26.2%, among the most inbred F_5 lineages (ANOVA, $P < 0.01$), intermediate (10.2%; NS) in the F_2 lineages, and least (1.5%) among the outbred F_0 lineages. After eight generations of selection, the among-lineage component of variance in mean pupal weight was not significant for any of

TABLE 6. Minimum variance estimates of heritability from the pooled Parent-Offspring regression and the intraclass correlation among full sibs for each generation and each level of inbreeding.

Generation	F_5		F_2		F_0	
	h^2	SE	h^2	SE	h^2	SE
1	0.178	0.080	0.263	0.085	0.333	0.088
2	0.190	0.081	0.281	0.086	0.382	0.090
3	0.125	0.078	0.156	0.079	0.156	0.079
4	0.117	0.077	0.225	0.083	0.287	0.086
5	0.178	0.080	0.203	0.082	0.415	0.091
6	0.229	0.083	0.161	0.079	0.264	0.085
7	0.041	0.081	0.269	0.089	0.322	0.088
8	0.313	0.087	0.232	0.083	0.300	0.083
Mean	0.171	0.031	0.224	0.018	0.308	0.035
Expected	0.157		0.266		0.367	

the inbreeding treatments nor were there any trends overtime in this variance component within selection treatments.

Overall, the results observed for the response to artificial selection for increased pupal weight are in accord with expectations from additive theory. The response to selection diminishes with the degree of inbreeding. The observed heritabilities also decrease with inbreeding as predicted by additive theory. Lastly, replicated lines within inbreeding treatments responded homogeneously to selection as might be expected for a short-term artificial selection experiment with large selection differentials and variance effective population numbers in the range of 70–80 breeding adults.

The Effects of Inbreeding and Selection on Fitness

The Effect of Inbreeding before Selection on the Mean and Heritable Variance in Offspring Numbers.

Mean number of adult offspring, AO, declined significantly with inbreeding ($F_0 > F_2 > F_5$, Kruskal-Wallis $H = 27.54$, $P < 0.001$; Table 7). From the census data, the fitnesses of the inbred adults relative to the population average fitness were calculated as the ratio of heterozygotes (+/b) to total population (+/b and b/b). These relative fitnesses were arcsine-square root transformed to achieve homoscedasticity for analysis of variance. Within the outbred treatment (F_0), the among-lineage variance was 4.51% of the total variance in relative fitness and not significantly different from zero. However, in the F_2 treatment, the among-lineage variance was 32.69% of the total ($0.025 < P < 0.050$) and, in the F_5 treatment, it was 21.42% of the total ($P < 0.001$). In both the F_0 and F_5 treatments, there was a significant effect of sex ($P < 0.05$ and $P \ll 0.001$, respectively). In fact, in the F_5 treatment, much of the among-lineage variation was exhibited

TABLE 7. Total fitness (A0) before selection and viability fitness after selection.

Treatment	Total fitness (A0) before selection		Survivorship (L) to 180 days			
	Mean	Variance	Control		Selection	
			Males	Females	Males	Females
F_0	44.47	406.08	0.65	0.10	0.71	0.07
F_2	29.68	451.33	0.61	0.03	0.52	0.05
F_5	13.92	720.01	0.60	0.04	0.39	0.08

TABLE 8. Decline in total fitness (TO) with inbreeding and selection relative to the mean of the unselected, outbred treatment (F₀ Control).

Generation	Inbreeding level					
	Controls			Selection		
	F ₀	F ₂	F ₅	F ₀	F ₂	F ₅
4	1.000	0.693	0.469	0.775	0.748	0.573
5	1.000	0.673	0.479	0.897	0.631	0.387
6	1.000	0.780	0.493	0.861	0.673	0.481
Mean	1.000	0.715	0.480	0.844	0.684	0.480

in the sex-lineage interaction variance ($P \ll 0.001$). In summary, the variance in relative fitness increased with inbreeding; the among-lineage variance in mean relative fitness was highest for the F₅ and F₂ treatments and lowest for the F₀ lineages. As discussed above, a similar pattern was found prior to selection for mean pupal weight where the most inbred lineages also exhibited the greatest among-lineage variance. There was, however, no significant correlation between mean relative fitness and mean pupal weight across lineages ($r = -0.172$, $df = 13$, $P > 0.30$).

The Effect of Inbreeding Level during Selection on the Mean and Heritable Variance in Offspring Numbers, TO

Mean total offspring per family (including larvae, pupae and adults), TO, was lower for the more inbred treatments than for the outbred treatment. In Table 8, the degree of this inbreeding depression for generations 4, 5, and 6, is expressed relative to outbred performance by dividing the observed mean offspring numbers per treatment by that of the outbred, unselected F₀ control population. Selection for increased pupal weight appears to have reduced mean fitness in the outbred populations (F₀) by an average of 15.6% relative to the unselected control. Comparing fitness (TO) in the selection treatments with that in the unselected controls, we find, for seven of nine comparisons, the mean TO is lower in the selection treatments (Table 8). (Note, however, that the observations in Table 8 are not independent across generations.)

In Table 9, we report the heritability of offspring number (TO) calculated from the census data in generations 4–6. Heritability was estimated as twice the average midparent-offspring regression for each level of inbreeding for both the selected and control treatments. In contrast with the data for pupal weight, there is no tendency for heritability in the more inbred treatments to be reduced relative to the outbred treatment. The value of heritability observed in F₅, the most inbred treatment, exceeds those of the other two treatments but the difference is not statistically significant.

The mean and variance of longevity, L

Average survivorship to day 180 (L) for males and females of each inbreeding level is presented in Table 7 for both the control and selection treatments. Analysis of variance of the arcsin square-root transformed survivorship data revealed significant effects of inbreeding level and sex ($P < 0.0001$ for each) but no significant variation among-lineages within inbreeding levels ($P > 0.20$) or between control and selection

TABLE 9. Average heritability of offspring numbers (TO) within lineages within inbreeding level for generations 5 and 6.

Treatment	Inbreeding level					
	F ₀		F ₂		F ₅	
	Mean	SE*	Mean	SE	Mean	SE
Selected	0.400	0.219	0.309	0.164	0.558	0.144
Control	0.441	0.144	0.252	0.077	0.505	0.183

* The standard errors (SE) are the empirical standard errors calculated across lineages and across generations.

treatments ($P > 0.42$). Overall, survivorship of males to 180 days was much higher than females and mean survivorship of both sexes declined with increased inbreeding. The lack of significant variation among-lineages within inbreeding levels is somewhat surprising given the evident inbreeding depression.

DISCUSSION

This paper contributes to the growing number of studies showing that inbreeding or population bottlenecks may affect the mean and variance of different traits in different ways. For the mainly additively determined trait, pupal weight, inbreeding diminished the heritable variance qualitatively and quantitatively in accord with the predictions of additive theory (Tables 5 and 6). Considering first mean values, fitness components exhibited evidence of classic inbreeding depression: mean fitness (AO) declined owing to inbreeding prior to selection (Table 7) and declined further (TO) with strong artificial selection (Table 8). Although mean fitness declined, the heritable variance in fitness within lineages did not (Table 9). The maintenance of heritable variance in fitness is of significance because it contributes to our understanding of speciation theories involving bottlenecks and to our understanding of selection in small natural populations. The overall interpretation, however, is ambiguous. It is difficult to interpret a decline in fitness with inbreeding optimistically with respect to the future evolutionary potential of the population. However, the lack of a decline in the heritable variance in fitness suggests that inbreeding and selection do not limit the adaptive potential of small populations. Thus, our results differ in some respects from expectations based on theoretical models that consider only additive genetic effects and neglect nonadditive genetic variation but they do not address the distinction between dominance and epistasis.

In our studies, we found that inbreeding prior to selection reduced the response to directional selection for increased pupal weight and the amount of the reduction agreed well with the expectations of additive genetic theory. Because each selected line was paired with an unselected control, we are able to investigate patterns of within-line genetic variance in the presence of among-line variation with more statistical power than previous studies. This is important because, in the absence of selection, replicate lines are expected to vary in within-line genetic variance (Avery and Hill 1977) making empirical tests of theoretical predictions inefficient (Katz and Enfield 1977). We also observed indirect effects of inbreeding and selection on the mean and variance of fitness components. Inbreeding prior to selection reduced mean total fitness (TO)

as expected and artificial directional selection appeared to further reduce it. These results are characteristic of other experimental studies of artificial selection in which progress under selection can be limited by a decline in fitness components, such as fertility (cf. Falconer 1989, p. 225). We did not observe, however, a decrease in the additive genetic variance for fitness even with high levels of inbreeding and directional selection (Table 9). Indeed, for some fitness components, the heritable variance was greatest in the most highly inbred and selected lines although not statistically so. In our study, life history parameters such as productivity and longevity appeared to respond differently than the morphological trait (pupal weight) to inbreeding and selection.

Some experimental work (notably, Bryant et al. 1986a,b; and Lopez-Fanjul and Villeverde, 1989; Meffert and Bryant 1992; Meffert 1995) suggests that inbreeding may affect heritable genetic variance in a manner not predicted by additive theory. For example, they found that the heritability of a number of continuous morphological traits (Bryant et al. 1986 a,b) and behavioral traits (Meffert and Bryant 1992; Meffert 1995) in *Musca domestica* increased after a population bottleneck. In *Drosophila melanogaster*, Lopez-Fanjul and Villaverde (1989) observed an increase in the heritable variance for viability after inbreeding. These authors emphasized the relationship of the traits to fitness and the possibility that nonadditive genetic variance was responsible for the findings. Unlike Bryant et al. (1986a,b), we found a decrease in heritable variance of pupal weight with inbreeding and selection. Similar to Lopez-Fanjul and Villaverde (1989), we found no change in the heritable variance in fitness (Table 9).

Our study also contributes to our understanding of selection in natural populations. Plant and animal breeders have long been interested in the problem of progress under selection for different mating systems (e.g., Lush 1947; Madalena and Hill 1972; Goodwill 1974; Katz and Enfield 1977). This is relevant to our study because inbreeding, bottlenecks and mating systems all can be used to address the relationship between the loss of heterozygosity and changes in genetic variance. Under certain genetic circumstances, non-random mating systems have been shown to increase the response to selection by changing levels of homozygosity (Wright 1921, 1952; Haldane 1937; Fisher 1949; Breese 1956; McBride and Robertson 1963; Wilson et al. 1965). Furthermore, different mating systems acquire homozygosity at different rates (Wright 1921). Our results for life history parameters are in agreement with these findings that increased inbreeding can be associated with an increase in the opportunity for selection.

Numerous experimental studies have examined the effects of mating system or inbreeding on selection for continuously varying traits in a variety of organisms (Tantawy and Reeve 1956; Wilson et al. 1965; reviews in Rathie and Nicholas 1980; Weir et al. 1980). Other empirical studies (Bowman and Falconer 1960; Madalena and Robertson 1975; Enfield 1976; Katz and Enfield 1977; Rathie and Nicholas 1980) have investigated the effects of population subdivision on the response to selection. In most of these experiments, artificial selection and inbreeding were imposed simultaneously to simulate the procedures used by animal breeders. The case of evolutionary interest to us, however, is one in which a

bottleneck or an episode of more or less intense inbreeding precedes selection. Thus, the empirical studies most relevant to these evolutionary questions are those that investigate the heritability of traits in a population after some period of inbreeding (Wright 1921, 1934; Tantawy and Reeve 1956; Bryant et al. 1986 a and b; Lopez-Fanjul and Villeverde 1989; Meffert and Bryant 1992; Meffert 1995).

Tantawy and Reeve (1956) observed that the heritability of wing length in *D. melanogaster* populations for most levels of inbreeding significantly exceeded the theoretical expectation for purely additive genetic effects and constant environmental variance. "It is obvious . . . that h^2 declines much more slowly than the theoretical curve with all inbreeding systems" (Tantawy and Reeve 1956, p. 659). They attributed this finding to selection against homozygosity during the course of inbreeding, although they acknowledged the possibility of some contribution from nonadditive gene effects.

In summary, our study is one of a number of recent studies that suggest population bottlenecks can enhance the potential of a population to respond to selection. Our findings for the morphological trait, pupal weight, support additive theory but our findings for fitness components suggest that different traits may respond differently to increased homozygosity resulting from inbreeding and selection.

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