

The effect of inbreeding on the redistribution of genetic variance of fecundity and viability in *Tribolium castaneum*

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Inbreeding was carried out to investigate the genetic properties of fecundity and viability (early and late) in a population of *Tribolium castaneum*. Heritability estimates in the base population were intermediate for fecundity and small and nonsignificant for viability. All traits showed strong inbreeding depression. For both viability traits, a significant increase of both within-line additive variance and between-line variance was observed with inbreeding. For fecundity, however, these changes were not detected. The results are compatible with the genetic variance of viability in the base population being generated by segregation of a number of deleterious recessive alleles at low frequencies. For fecundity, an additional class of loci with substantial additive effects must be postulated.

Keywords: artificial selection, fecundity, genetic variance, inbreeding, *Tribolium castaneum*, viability.

Introduction

The redistribution of the genetic variance of a neutral quantitative trait in a population subdivided into lines of equal effective size has been theoretically studied for different types of gene action. The cases of additive genes, recessives at low initial frequency and additive \times additive epistasis have been considered by Wright (1951), Robertson (1952) and Goodnight (1988), respectively.

Summarizing, with additive action within and between loci, the between-line variance relative to the genetic variance in the base population increases linearly with the inbreeding coefficient (F_t) as $2F_t$. The increase will be larger than that predicted by the additive model when part or all initial variation is caused by rare recessive alleles and/or by loci showing additive \times additive epistasis. In parallel, the within-line additive genetic variance contributed by additive loci, also expressed as a proportion of the base population value, is expected to decrease with time as $(1 - F_t)$. In the other cases, however, it can initially increase with inbreeding up to a maximum,

and subsequently decrease to zero. Thus, genetic drift can convert nonadditive variance into additive variance which may contribute to the response to selection (Goodnight, 1987, 1988; Tachida & Cockerham, 1989). This phenomenon is particularly relevant to the efficacy of the interdemic selection phase of Wright's shifting balance theory of evolution (Wright, 1978; Wade & Goodnight, 1991) and to the response to artificial selection in structured populations (see López-Fanjul, 1989, for a review).

Experimental data supporting this conversion of nonadditive into additive variance are scarce (see Carson, 1990, for a review). In *Musca domestica*, Bryant *et al.* (1986) found an increase of the additive variance of several morphological traits after lines passed through a single-generation bottleneck. The statistical significance of this result has been questioned by Lynch (1988). In *Drosophila melanogaster*, López-Fanjul & Villaverde (1989) and García *et al.* (1994) obtained a significant increase of both the additive variance and the heritability of egg-to-pupa viability after one generation of brother-sister mating.

The objective of the present experiment is to explore the situation for life-history traits in *Tribolium castaneum*, following an experimental design

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analogous to that used by López-Fanjul & Villaverde (1989).

Materials and methods

The Consejo population was captured near Madrid and has been maintained in this laboratory since 1964 in ten bottles. A circular mating scheme was used to ensure a sufficiently large population (about 1000 potential parents per generation). All beetles in this experiment were kept in the same dark incubator at 70 per cent relative humidity and 33°C. The culture medium consisted of 95 per cent whole wheat flour and 5 per cent dried powdered brewer's yeast by weight.

Three fitness component traits were considered: (1) 2-day egg-laying of mated females, individually scored from the 11th to the 13th day after adult emergence; (2) early viability, measured individually as the ratio between the number of eggs laid by a female during the period mentioned and the number of larvae present 2 days later; and (3) late viability, measured in the same way as for early viability but with respect to the number of larvae present 11 days later. Both viability traits were expressed in percentages.

Thirty-two lines, 16 inbred ($F_0 = 0.25$) and 16 non-inbred ($F_0 = 0$) were established. For the three traits, the mean, between-line variance and within-line additive genetic variance in both types of lines were compared.

Noninbred lines

From the Consejo population, a sample of 160 males and 160 virgin females was collected, mated at random in pairs, and divided into 16 groups of equal size. From each group, both a selected and a control line were started, these treatments being assigned at random with the restriction of there being eight lines of each type. One generation of upward selection was carried out independently for each of the three traits considered. To do this, three sets of two male and two female offspring were obtained from each of the 10 original pairs in a group. In each set, 20 random matings were established and a different trait scored (generation 0). The five highest scoring pairs were selected as parents and each contributed four male and four female offspring. These 20 males and 20 females were mated at random and the resulting pairs were evaluated for the selected trait (generation 1). The control lines were maintained in the same way as the selected lines, but the three traits were evaluated on the same 20 matings per

generation, and the five pairs of parents were randomly selected. Full-sib matings were always avoided in all lines and in all generations.

Inbred lines

Eight matings were randomly taken from the base population. From each mating, five offspring of each sex were taken and mated individually, each sib mating contributing 16 male and 16 female offspring. These 80 pairs were divided into four sets of 20 pairs each. From each set, a line selected for one of each of the three traits considered and a control line were started. From here, we followed the same procedure as in the noninbred lines.

In all, there were eight inbred and eight non-inbred lines selected for each trait, along with their corresponding randomly selected control lines. All lines selected for the same trait (inbred or non-inbred) and their companion control lines were maintained on a strictly contemporary schedule.

Divergent selection for fecundity or viability is expected to show negative asymmetry, as downward response will be mostly the result of an increase in the frequencies of all sorts of deleterious alleles present in the base population. Therefore, downward selection was not carried out in our experiment, as the response obtained would not be relevant to our purposes.

For each selected line and trait the following parameters were calculated: (1) selection differential (S), as the difference between the mean of the selected parents and that of the population from which they were taken; (2) response to selection (R), as the difference between the means of the selected trait at generations 1 and 0, expressed as deviation from that of the companion inbred control line or from the average of all noninbred control lines; (3) realized heritability (h_R^2), as the quotient between the response and the selection differential; (4) phenotypic variance (V_{P0}) at generation 0; and (5) additive genetic variance (V_{A0}) at generation 0, as the product of the realized heritability by the phenotypic variance. Standard errors of average phenotypic and additive variances over lines were estimated empirically. Those of the between-line component of variance were calculated following the procedure outlined by Lynch (1988). Only calculations on untransformed data are presented as the conclusions were not substantially modified after an arcsine transformation was performed.

Avoidance of full-sib mating (except in the formation of the inbred lines) ensured that the rate of

inbreeding was negligible during the period considered (less than 0.01 per generation) both in the control and the selected lines. Thus, the inbreeding coefficient of selected parents and their offspring was approximately the same. This was deemed important, as viability can in principle be regarded as a property both of the mother and of the offspring. In *Drosophila melanogaster*, viability seems to be largely dependent on the maternal genotype (see Chapco, 1979, for a review), but this property has not been investigated in *Tribolium*. We have considered viability as a trait of the mother, which implies that we were carrying out individual selection. On the other hand, if viability had depended entirely on the larval genotype, family selection would have been practised and the realized heritability would have been the heritability of full-sib family means. This does not affect the significance of the difference between the realized heritabilities estimated in inbred and noninbred selected lines, as this significance has been tested empirically.

Results

Table 1 shows the effects of inbreeding on the mean, and on the within- and between-line components of the phenotypic variance for egg-laying and both viability traits in the control lines.

In each type of control line (inbred and noninbred), the difference between the means at genera-

tions 0 and 1 was not significant for all traits. However, significant differences were found between the means of inbred and noninbred control lines ($P < 0.05$). The rate of inbreeding depression per 10 per cent increase in inbreeding coefficient was 2.6 eggs, 1.7 larvae and 4.2 larvae for fecundity, early viability, and late viability, respectively (about 4–5 per cent of the noninbred mean). On the average, both the within- and the between-line components of the phenotypic variance considerably increased with inbreeding for all traits. Increments in the within-line variance were significant in all cases; those corresponding to the between-line variance were only significant for late viability ($P < 0.05$).

Table 2 shows the average values of S , R , V_P , h_R^2 and V_A for each trait and type of line. For fecundity, all those values were significantly different from zero ($P < 0.05$) both in inbred and noninbred lines. However, in no case was a significant difference detected between these two sets of lines. On the other hand, the behaviour of both viability traits was very similar and strikingly different from that observed for fecundity. The average selection differential was higher in inbred than in noninbred lines, reflecting a parallel increase in the phenotypic variance. Selection resulted in a negative response on average in the noninbred lines (not significantly different from zero). However, the response to selection in the inbred lines was positive and significant for both traits. Consequently, the additive genetic variance of

Table 1 Mean, within-line (V_W) and between-line (V_B) components of the phenotypic variance for fecundity and viability of randomly selected control lines of *Tribolium castaneum* in two consecutive generations (t)

Trait	Type of line	t	Mean	V_W	V_B
Fecundity	Noninbred	0	44.3 ± 0.6	46.1 ± 5.5	1.1 ± 1.9
		1	43.1 ± 0.5	36.0 ± 4.5	3.6 ± 3.0
		Average	43.7 ± 0.4	41.1 ± 3.6	2.4 ± 1.8
	Inbred	0	37.1 ± 0.7	72.9 ± 8.8	3.4 ± 3.9
		1	37.3 ± 0.6	55.6 ± 6.7	6.1 ± 4.9
		Average	37.2 ± 0.5	64.3 ± 5.5	4.8 ± 3.2
Early viability	Noninbred	0	41.4 ± 0.7	78.5 ± 9.4	3.1 ± 3.9
		1	41.7 ± 0.8	86.7 ± 10.8	4.5 ± 5.0
		Average	41.6 ± 0.5	82.6 ± 7.2	3.8 ± 3.2
	Inbred	0	36.6 ± 1.0	149.1 ± 17.9	13.3 ± 11.4
		1	38.0 ± 0.8	88.8 ± 10.7	16.6 ± 11.4
		Average	37.3 ± 0.7	119.0 ± 10.4	15.0 ± 8.1
Late viability	Noninbred	0	80.9 ± 1.1	191.5 ± 23.1	7.7 ± 6.7
		1	77.3 ± 1.5	308.9 ± 38.5	3.5 ± 11.3
		Average	79.1 ± 0.9	250.2 ± 22.4	5.6 ± 7.6
	Inbred	0	70.1 ± 1.6	350.4 ± 42.0	44.2 ± 33.8
		1	67.1 ± 1.5	294.9 ± 35.4	43.7 ± 31.9
		Average	68.6 ± 1.1	322.7 ± 27.5	44.0 ± 23.3

Table 2 Average selection differential applied (S), response (R), phenotypic (V_{P0}) and additive (V_{A0}) variances of the base population of *Tribolium castaneum*, and realized heritability (h_R^2) of the noninbred ($F_0 = 0$) and inbred ($F_0 = 1/4$) selected lines

	Trait					
	Fecundity		Early viability		Late viability	
	$F_0 = 0$	$F_0 = 1/4$	$F_0 = 0$	$F_0 = 1/4$	$F_0 = 0$	$F_0 = 1/4$
S	$8.0 \pm 0.9^*$	$8.6 \pm 0.5^*$	$9.9 \pm 1.0^*$	$11.6 \pm 1.4^*$	$11.6 \pm 1.1^*$	$19.4 \pm 2.4^{*\dagger}$
R	$4.7 \pm 0.6^*$	$3.7 \pm 2.2^*$	-2.1 ± 1.1	$4.7 \pm 1.7^{*\dagger}$	-1.4 ± 1.8	$7.3 \pm 2.4^{*\dagger}$
V_{P0}	$40.2 \pm 9.6^*$	$46.8 \pm 10.1^*$	$98.9 \pm 22.5^*$	$114.1 \pm 27.3^*$	$158.8 \pm 24.9^*$	$283.3 \pm 36.9^{*\dagger}$
V_{A0}	$20.8 \pm 2.4^*$	$18.8 \pm 8.4^*$	2.9 ± 2.3	$42.9 \pm 12.5^{*\dagger}$	13.0 ± 7.6	$107.7 \pm 31.7^{*\dagger}$
h_R^2	$0.50 \pm 0.20^*$	$0.40 \pm 0.10^*$	0.03 ± 0.02	$0.40 \pm 0.10^{*\dagger}$	0.08 ± 0.05	$0.40 \pm 0.10^{*\dagger}$

*Significantly different from zero ($P < 0.05$, t -test).

†Significantly different from estimate at $F_0 = 0$ ($P < 0.05$, t -test)

Table 3 Observed (O) values of the change of the within-line additive variance (V_{AF}) and the between-line variance (V_{BF}) with $F = 1/4$ inbreeding, together with expectation (E) for n_1 additive and n_2 equivalent recessive loci initially segregating at frequencies p_1 and p_2 , respectively, generating the additive variance in the base population of *Tribolium castaneum* ($F = 0$) and the inbreeding depression observed

	n_1	n_2	p_1	p_2	V_{AF}/V_{A0}		$(V_{BF} - V_{B0})/V_{A0}$	
					O	E	O	E
Fecundity	24	43	0.50	0.10	0.90	0.91	0.12	0.57
	25	135	0.07	0.07	0.90	0.89	0.12	0.55
Early viability	—	29	—	0.07	14.79	13.80	3.86	5.83
Late viability	—	29	—	0.10	8.28	7.58	2.95	3.36

both viability traits was significantly larger in the inbred lines ($P < 0.01$). As heritability values should lie between 0 and 1, anomalous estimates were set to the nearest bound. This procedure results in biased estimates of the average realized heritabilities and their sampling errors are reduced (Toro & Prunionsa, 1984). Adjusted estimates are shown in Table 2. Both additive genetic variances and heritabilities were significantly larger in the inbred lines ($P < 0.01$). This result was unaffected when the calculations were based on unadjusted estimates.

The distributions of the within-line additive variance in inbred and noninbred lines are shown in Fig. 1 for each trait. For viability traits, little overlap between these distributions was observed.

Discussion

Our experimental results can be conveniently divided into two parts, according to the character

considered. For both viability traits, the following were observed: (1) considerable inbreeding depression, (2) a very small heritability at $F_0 = 0$, (3) a drastic rise of the within-line additive variance with inbreeding, and (4) a parallel increase of the between-line variance larger than predicted by the additive model. These results are consistent with the genetic variance of viability in the base population being essentially generated by deleterious recessive alleles at low frequencies.

Fecundity was also depressed with inbreeding, but the remaining variables behave in a completely different manner, showing: (1) an intermediate heritability at $F_0 = 0$, and (2) small nonsignificant changes of the within-line additive variance and the between-line variance with inbreeding. For this trait, it appears that both deleterious recessive alleles and loci with substantial additive effects are segregating in the base population.

In order to obtain a better understanding of the situation, consider the following numerical example.

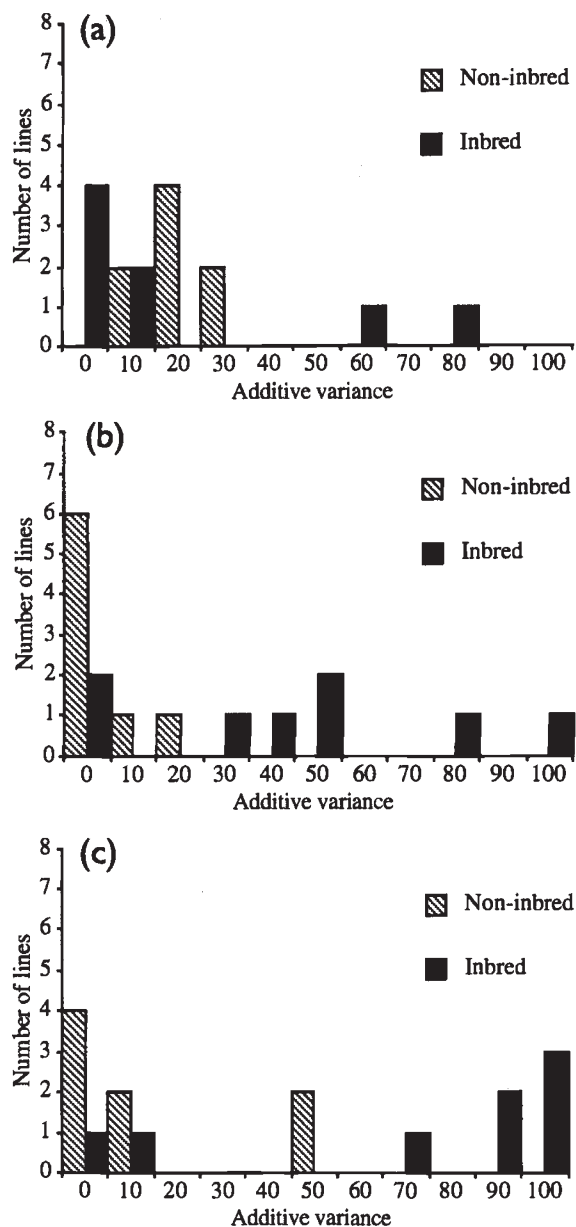


Fig. 1 Distribution of the additive genetic variance in the inbred and noninbred lines of *Tribolium castaneum* for (a) fecundity, (b) early viability and (c) late viability.

Assume n_1 additive and n_2 completely recessive biallelic loci, all of the same effect ($2a$) and frequencies p_1 and p_2 , equal for all loci in each class, respectively. Expected values of the initial additive variance (V_{A0}) and the inbreeding depression D at $F = 1/4$ are given by (Falconer, 1989)

$$V_{A0} = n_1(2a^2p_1q_1) + n_2(8a^2p_2q_2^3) \quad (1)$$

and

$$D = n_2ap_2q_2/2. \quad (2)$$

Values satisfying eqns 1 and 2 for the observed V_{A0} and D values and also giving a good fit between expected values at $F = 1/4$ (Crow & Kimura, 1970) and those obtained for the within-line additive (V_{AF}) and between-line (V_{BF}) variances, both expressed as proportions of the additive variance at $F = 0$, are given in Table 3 together with the corresponding observations. The number of loci involved (n_1 and n_2) is inversely proportional to the value of a . Those in Table 3 have been calculated for $a = \sqrt{V_{P0}/2}$.

Thus, 29 equivalent recessive loci with standardized effect equal to one and the recessive allele segregating at low initial frequency, satisfactorily explain the viability results. For fecundity, however, a number of additive loci at intermediate frequencies has to be assumed in addition to deleterious recessives at low frequencies. Alternatively, a comparable fit can be obtained for small frequencies at both classes of loci, implying a large increase of n_2 . Of course, this numerical example only shows the internal coherence of our qualitative interpretation of the data and does not provide quantitative estimates of relevant parameters.

Epistasis could also result in an increase of the within-line additive variance with inbreeding (Goodnight, 1988). Although we unequivocally detected dominance effects from inbreeding depression, our experimental design does not allow a separate study of epistatic effects. Therefore, this discussion has been framed in the simplest terms, i.e. in the absence of nonallelic interactions.

In *Tribolium castaneum*, previous estimates of relevant parameters for fecundity and viability have only been reported for the Consejo population (Orozco & Tagarro, 1969; López-Fanjul & Jódar, 1977) and are in good agreement with those obtained in the present experiment. Thus, both traits showed inbreeding depression. Furthermore, the heritability of viability was small and nonsignificant (0.17 ± 0.09) and that of fecundity ranged between 0.14 and 0.54. However, these values are likely to be overestimates as they have been obtained from full-sib correlation analysis. Therefore, in our numerical example the relative contribution of additive loci may be inflated if our estimate of heritability can be considered abnormally high.

In *Drosophila melanogaster*, inbreeding and artificial selection experiments have also been carried out to investigate the genetic properties of fecundity and viability (Rose, 1984; López-Fanjul & Villaverde, 1989; García *et al.*, 1994). For both traits, the results are compatible with the genetic variance in populations being generated by segregation at a few loci

with substantial additive effects and a number of deleterious recessives at low initial frequencies.

Finally, the relevance of the observed redistribution of the genetic variance with inbreeding must be considered. In the inbred lines, higher responses to selection for increasing viability were obtained, but they were accompanied by only a partial recovery (about 50 per cent) of the inbreeding depression incurred. Thus, the significance of this phenomenon on the outcome of natural or artificial selection is uncertain. On the other hand, the observed increment of the between-line variance with inbreeding emphasizes the greater evolutionary potential of small populations postulated by the shifting balance theory. The development of artificial selection strategies making efficient use of the conversion of nonadditive variance into additive variance by means of inbreeding requires further investigation and experimental validation (see Toro, 1993, for a review).

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