

THE GENETIC PROPERTIES OF EGG LAYING OF VIRGIN FEMALES OF *TRIBOLIUM* *CASTANEUM*

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SUMMARY

Inbreeding was established by brother-sister matings and it was carried out for seven generations in the Consejo population of *Tribolium castaneum*. From 105 inbred lines at the start, 53 lines reached the end of the experiment. Losses of lines were independent of the level of inbreeding.

The trait studied was 4-day egg laying of virgin females. It has been shown that this trait did not present either inbreeding depression or hybrid vigour. The phenotypic variance and its between- and within-line components also behaved as expected under the additive model as inbreeding progressed. Estimation of genetic parameters in the base population confirmed that egg laying of virgin females is an essentially additive trait.

Other traits—2-day egg laying of fecundated females, precocity and percentage viability—showed a smaller heritability than egg laying of virgin females and considerable inbreeding depression. No connection was found between virgin female egg laying and percentage viability.

The suggestion is made that egg laying of virgin females in the Consejo population can be considered a peripheral trait with respect to fitness.

1. INTRODUCTION

POPULATIONS are constantly subjected to the action of natural selection tending to increase fitness. Fisher's (1930) fundamental theorem of natural selection states that the rate of increase of the reproductive fitness of a population at any given moment is directly proportional to the additive genetic variance of fitness of the population at that moment. Therefore, a population at genetic equilibrium should have very little or no additive variance for fitness. The genetic properties of a quantitative trait in a population, as we see them at present, must be the product of the action of natural selection in the past through a functional relationship between the trait and fitness itself (Robertson, 1955). The secondary theorem of natural selection states that the rate of change of the mean of a metrical character is proportional to the additive genetic covariance between the character and fitness (Robertson, 1968).

Experimentally, the problem of determining the genetic properties of a quantitative trait can be approached either by chromosome assay, a technique practically restricted to *Drosophila* (Kearsey and Kojima, 1967), or by statistical analysis of the genotypic variation. In the latter case, the ratio between the additive and the total genetic variances can be used as an indication of the closeness of the relationship between the trait and fitness.

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This ratio is expected to decrease as the relation becomes closer (Falconer, 1960). Unfortunately, the total genetic variance for a given trait is very difficult to estimate in a panmictic population. On the other hand, as inbreeding depression and its converse hybrid vigour are related to a proportion of the non-additive genetic variance, traits showing greater inbreeding depression are expected to be those more closely connected to fitness. Thus, the fundamental and the secondary theorems of natural selection provide the framework within which the relationship between a quantitative trait and fitness can be discussed.

The present experiment follows the approach outlined above in the study of the genetic architecture of egg laying of virgin females in a long-established laboratory population of *Tribolium castaneum*, a trait which might be considered at first sight as one related to fitness. Results from a preliminary experiment have been already reported by Zornoza and López-Fanjul (1975).

2. MATERIAL AND METHODS

The Consejo population is a pool of several wild populations captured near Madrid and has been maintained in cages in this laboratory at 33°C (Orozco and Bell, 1974a).

The traits considered were: (1) 4-day egg laying of virgin females scored from the 7th to the 11th day after adult emergence; (2) 2-day egg laying of fecundated females scored from the 11th to the 13th day after adult emergence; (3) precocity, measured individually as the offspring number of larvae, pupae and adults scored after 21 days from mating; (4) percentage viability, measured individually as the ratio of the number of adult offspring present after 40 days from mating to the number of eggs laid by the fecundated female during the 2-day period mentioned above.

All lines in this experiment were kept at 70 per cent relative humidity and 33°C, except for egg laying tests which were carried out either at 33°C or 28°C. The culture medium consisted of 95 per cent whole wheat flour and 5 per cent dried brewer's yeast.

From a large sample of pupae of each sex from the population cage, two random samples (I and II) of 110 males and 110 females each were taken and individually mated at random in vials within each sample. From sample I, 105 inbred lines were established by brother-sister single pair matings and continued for eight generations. These lines were maintained by a single pair chosen at random every generation; only if this pair reproduced did the line survive, no substitutions having been made. From sample II, a control line was established and it was reproduced each generation by 100 single pair random matings, each contributing one offspring of either sex to the next generation. When sterile matings occurred, additional matings were made in the next generation in an attempt to maintain the number of families in the control line at 100. As all females had been scored for virgin egg laying prior to mating, when a line or family were lost infertility necessarily arose after this period. It has not been possible from our data to ascribe the loss of a line (family) to male or female infertility separately.

Egg laying tests were made every generation at 33°C and 28°C on samples of about 16 virgin females each per inbred line and of about five virgin females from each family of the control line, respectively. At generation 7

of inbreeding, precocity and percentage viability were measured in both the surviving inbred lines and the control line. Both inbred and control lines were contemporaries during the whole experiment.

3. RESULTS

(i) *The base population*

The parameters of the base population for the four characters studied are given in table 1, together with other heritability estimates for egg laying of virgin females at the two temperatures considered, already reported for the Consejo population. Both mean and variances of virgin female egg laying were higher at 33°C.

TABLE 1

Parameters of the base population for egg laying of virgin females at 33°C (LV33) and 28°C (LV28), egg laying of fecundated females at 33°C (LF) and percentage viability at 33°C (% V)

	LV33	LV28	LF	% V
Mean	22.31	15.11	28.65	75.19
Standard deviation	0.27	0.19	0.43	0.87
Phenotypic variance	209.73	101.66	82.02	295.93
Coefficient of variation (%)	65	67	32	23
Variance components:				
Within-family	169.06	80.20	70.19	269.57
Between-family	32.21	16.08	11.83	26.36
Heritability \pm S.E.:				
Full-sister correlation	(a) 0.31 \pm 0.04	0.32 \pm 0.04	0.29 \pm 0.09	0.17 \pm 0.09
	(b) 0.38 \pm 0.04	0.33 \pm 0.04	—	—
	(c) 0.30 \pm 0.06	—	—	—
	(d) 0.38 \pm 0.04	0.33 \pm 0.04	—	—
Daughter-dam regression	(a) 0.30 \pm 0.06	—	0.14 \pm 0.12	—
	(b) 0.37 \pm 0.02	0.27 \pm 0.02	—	—
	(c) 0.27 \pm 0.02	—	—	—
	(d) 0.36 \pm 0.03	0.26 \pm 0.03	—	—

(a) Present work; (b) Orozco and Bell (1974a); (c) Ruano, Orozco and López-Fanjul (1975); (d) Orozco (1976b).

Heritabilities have been calculated by full-sister correlation and daughter-dam regression analyses. Twice the difference between these heritabilities estimates the relative importance of the dominance and maternal effects plus a fraction of the epistatic effects (Dickerson, 1959). For egg laying of virgin females at 33°C this difference was 0.02 with an approximate standard error of 0.14. This estimate is in very good agreement with others obtained at 33°C (table 1) indicating the essentially additive nature of this trait in the Consejo population. A regression estimate of the heritability of egg laying of virgin females at 28°C could not be obtained from our data. Significant differences between the correlation and the regression estimates of the heritability of this trait have been obtained by Orozco and Bell (1974a) and Orozco (1976b) who interpreted them as evidence of dominance gene action for this trait.

Although fecundated females were only allowed a laying period of 2 days, they laid more eggs than virgin females, in agreement with the results obtained by Orozco and Tagarro (1969). Both egg laying of fecundated females and percentage viability show a smaller heritability than egg laying of virgin females. Estimates of the genetic and phenotypic correlations between egg laying at 33°C of virgin and fecundated females in the base population were obtained by full-sister analysis of variance and covariance. Those estimates were 0.41 ± 0.12 and 0.19 ± 0.09 , respectively.

(ii) *The inbreeding results for egg laying of virgin females*

The structure of control and inbred lines is shown in table 2. From 100 pair matings set up every generation in the control line, the average loss amounted to 11 per cent families per generation. Using this figure as an estimate of loss we would expect 52 inbred lines to survive after seven generations of inbreeding, in extremely good agreement with the 53 lines

TABLE 2
Structure of control (C) and inbred (I) lines at different generations

Generation of inbreeding	Number of families (lines) scored		Average number of individuals scored per family (line)	
	<i>C</i>	<i>I</i>	<i>C</i>	<i>I</i>
1	99	105	4.69	13.86
2	86	99	4.23	14.02
3	79	89	4.91	20.22
4	91	83	4.89	19.22
5	89	73	4.25	14.11
6	90	60	4.77	13.57
7	91	53	4.84	15.66

which actually reached the end of the experiment. Losses of inbred lines appear then to be independent of the inbreeding process. Moreover, no significant differences were found between the frequency distributions of egg laying at both temperatures for all inbred lines and for those surviving after seven generations of inbreeding ($P > 70$ per cent). Similarly, the differences between the distributions of the means for all inbred lines and for those surviving were also non-significant at any temperature ($P > 80$ per cent).

The means, phenotypic variances and variance components of both control and inbred lines are shown in table 3. Inbred lines considered as a whole and those surviving both behave in a similar manner. In consequence, only data corresponding to the latter lines are shown. Strong fluctuations of these parameters occurred throughout the experiment unassociated with any known culture change. Higher means tended to correspond to higher variances probably due to the positive correlation existing between the mean and the variance for the traits studied. The data were left untransformed as no appropriate scale transformation has been found rendering the mean and the variance uncorrelated (Orozco, 1976a). The differences between the means of control and inbred lines at the two temperatures showed no specific tendency during the entire experiment, indicating the absence of

inbreeding depression. At 33°C, the differences between the control and the inbred lines for the phenotypic and the within-line variance did not follow any specific trend as inbreeding progressed. For egg laying at 28°C, the values of these parameters were in general lower in the control line. The between-line variance at both temperatures shows a clear-cut increase as inbreeding progresses. This variance was always larger than the variance between families of the control line in all generations.

Inbred lines were random crossed in pairs at generation 6 and 50 F_1 progeny from 22 out of the 26 possible crosses being obtained and scored at 33°C. In spite of large differences between the means of the reciprocal F_1

TABLE 3

Means and phenotypic, within-family (line) and between-family (line) variances of control (C) and inbred (I) lines for each level of inbreeding (F)

F	Mean		Phenotypic variance		Within-family variance		Between-family variance	
	C	I	C	I	C	I	C	I
(a) Egg laying of virgin females at 33°C								
0.250	19.4	18.9	162.8	144.6	139.6	120.9	23.2	23.7
0.375	19.7	22.3	151.6	222.4	133.2	175.6	18.4	46.8
0.500	27.2	22.7	203.5	185.1	172.0	147.6	31.5	37.5
0.594	22.9	23.5	189.0	246.7	170.7	183.3	18.3	63.4
0.672	18.9	21.7	162.8	225.9	135.1	155.6	27.7	70.3
0.734	24.4	19.9	233.8	195.5	182.2	139.5	51.6	56.0
0.785	23.6	20.1	294.6	227.5	239.5	158.2	55.1	69.3
(b) Egg laying of virgin females at 28°C								
0.250	11.0	12.4	78.7	83.7	70.1	72.2	8.6	11.5
0.375	14.3	17.4	89.6	133.2	76.8	106.5	12.8	26.7
0.500	18.7	17.3	111.5	127.0	94.2	97.2	17.3	29.8
0.594	15.8	16.8	81.1	116.8	69.0	87.9	12.1	28.9
0.672	15.4	14.0	111.8	104.3	95.6	80.6	16.2	23.7
0.734	16.5	16.0	100.9	135.4	73.4	87.8	27.5	47.6
0.785	14.5	16.3	104.2	118.2	86.5	80.3	17.7	37.9

crosses, the differences between the F_1 means and the corresponding mid-parental values were only found to be significant at the 5 per cent level in two cases. The regression coefficient of crossbred (F_1) mean on mid-parental mean was estimated to be 1.03 ± 0.27 .

(iii) *The effect of inbreeding on other traits*

The performance of the control and the inbred lines for egg laying of fecundated females, precocity and egg to adult viability was examined at the end of the experiment. These three characters presented considerable inbreeding depression (table 4), indicating the presence of non-additive genetic effects. This result also shows that the inbreeding process carried with it an increase in the level of homozygosity in spite of the absence of inbreeding depression for egg laying of virgin females.

The relationship between egg laying of virgin females and other traits—egg laying of fecundated females and percentage viability—was also investi-

gated both in the control and the inbred lines at generation 7 and the results are presented in table 5. A significant positive association was found between virgin and fecundated female egg laying, although the measure of the association indicates only a small degree of dependence (genetic correlation 0.41). On the other hand, the trait more closely connected to fitness of all traits studied—percentage viability—was found to be unassociated with egg laying of virgin females at 33°C ($P < 5$ per cent). In no case was an indication found of the existence of a favoured intermediate phenotype for egg laying. Unfortunately, the connection between egg laying of virgin females and precocity could not be investigated with our data.

TABLE 4

Means (\pm S.E.) of control (C) and inbred lines (I) at generation 7 of inbreeding for egg laying of fecundated females (LF), percentage viability (% V) and precocity (P) (a)

Line	LF	% V	P		
			% larvae	% pupae	% adults
C	28.65 \pm 0.43	75.19 \pm 0.87	10	88	2
I	21.37 \pm 0.37	44.42 \pm 0.83	45	55	0

(a) Based on about 400 and 700 individuals scored for the C and I lines, respectively for all traits.

TABLE 5

Egg laying of fecundated females (LF) and percentage viability (% V) for the different classes of egg laying of virgin females at 33°C (LV33), for the control (C) and the surviving inbred (I) lines (a)

	Class interval (LV33)						
	0-9	10-19	20-29	30-39	40-49	50-59	> 60
LF $\left\{ \begin{array}{l} C \\ I \end{array} \right.$	24.67	26.95	29.39	30.54	31.67	36.06	38.45
	17.58	20.47	23.99	26.01	28.51	27.94	31.94
% V $\left\{ \begin{array}{l} C \\ I \end{array} \right.$	76.36	74.53	74.06	76.45	74.46	73.34	74.70
	43.30	45.08	45.92	46.02	40.39	42.45	36.82

(a) Based on about 400 and 700 individuals scored for the C and I lines, respectively for all traits.

4. DISCUSSION

In a model of complete additive gene action, a trait subjected to inbreeding must not show either inbreeding depression or hybrid vigour. Besides, the changes in the total genetic (additive) variance and variance components for a set of lines all inbred by an amount F can be predicted in terms of their original values in the base population under panmixia ($F = 0$) and their inbreeding coefficient. The within-line genotypic variance decreases by a factor of $(1 - F)$, the between-line genotypic variance changes by a factor of $2F$ and the total genotypic variance increases by a factor of $(1 + F)$. This prediction is strictly true only when inbreeding is slow but with rapid inbreeding similar results can be expected except in the first few generations (Wright, 1951). In our case, the character egg laying of virgin females can be said to fulfil essentially all these conditions. Of course, for a trait

with a heritability of 0.30 and a coefficient of variation of about 65 per cent, the expected changes in the total genotypic and within-family genotypic variance would mostly go undetected as they have to be inferred from their corresponding phenotypic values which will, in turn, fluctuate widely. On the other hand, changes in the between-line variance will be easier to detect as this parameter is free of environmental effects. This has actually happened in our experiment.

For the above predictions to be valid two more assumptions have to be met. In the first place, the environmental variance should not change with inbreeding. This appears to be the case as the difference between the phenotypic variances in the control and the inbred lines does not follow any specific trend throughout the experiment. Secondly, there should be no selection for the trait considered at any stage. This also seems to be the situation as no differences were detected between the distributions of the trait in inbred lines as a whole and in those surviving. We can therefore conclude that egg laying of virgin females at 33°C and 28°C is essentially an additive trait within the power of resolution of our techniques. This conclusion does not preclude the existence of loci showing non-additive gene action for the trait but the gene frequencies of these loci in the base population should be low enough not to be detected by inbreeding, although these frequencies may be increased by recurrent selection (Orozco and Bell, 1974b).

Under the circumstances described, natural selection should have acted on the trait in the way described by Fisher's theorem provided a relationship between the trait and fitness exists. This is in contradiction with the presence of a large additive component of the genetic variance for egg laying of virgin females. Alternatively, centripetal selection could result in an essentially additive trait (Mather, 1953). Although our evidence is limited to one major component of fitness—percentage viability—no relation could be detected between this trait and egg laying, either in the control or in the inbred lines, in spite of the strong modifications of the gene frequency array which should be carried out by the inbreeding process.

However, it is quite possible that the selection pressure on the trait may be small and consequently its effect will not be detectable, making it impossible to differentiate a strictly neutral trait with respect to fitness from a peripheral trait, which lies outside the path linking primary gene action to reproductive fitness (Robertson, 1955). Judging from our data, egg laying of virgin females can then be considered a peripheral trait with respect to fitness in the Consejo base population. On the other hand, Orozco (1972) has shown that the peripheral nature of this trait disappears when the frequency array of the genes controlling virgin egg laying is modified by long-term artificial selection, which alters the position of the trait in relation to fitness.

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