

Long-term Selection for a Quantitative Character in Large Replicate Populations of *Drosophila melanogaster*. V.* The Inbreeding Effect of Selection

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Abstract

Four replicate lines of *D. melanogaster*, which had been selected for increased abdominal bristle number for 58 or 69 generations, were pedigreed for nine generations under selection with or without replacements for failed matings (SW and SO sublines) and under relaxed selection also with or without replacements (RW and RO sublines).

Natural selection was effective in reducing mean bristle number in both RW and RO sublines (except in one line), but its opposition to artificial selection in SW and SO sublines appeared to be only indirect. The relation between the two selective forces was inferred from their effects on effective population size, the comparison of selection responses in SW and SO sublines, and the difference between expected and realized selection differentials.

Fertility was the most important factor affecting variance effective population size, while fecundity (with upper limits on the number of offspring scored) and artificial selection were in most sublines similar in their relative importance. Measured by reduction in effective population size, the inbreeding effect of artificial selection confined to the immediate generation was small (6.4%), but the cumulative effect estimated from the observed rate of inbreeding was quite large (16%), tending to increase with more response.

The spread of genes from initial families seems to have been influenced by both artificial and natural selection. Correlations between the mean score of an initial family and its genetic contribution indicate that additive genetic variance was still available at this stage of selection. The number of initial families represented in SW and SO sublines was generally large even after nine generations, and few families made unusually large contributions.

Introduction

In an earlier paper in this series (Yoo 1980), the long-term behaviour has been described of large replicate lines of *Drosophila melanogaster* selected for increased abdominal bristle number in terms of selection response and variation among replicates. In such lines, deterioration of reproductive fitness resulting from long-continued selection often causes variation in progeny number beyond experimenters' control even with *D. melanogaster*, probably reducing effective population size (Kimura and Crow 1963) considerably below the actual number of parents used in each generation. On the other hand, reduction in effective population size due to artificial selection (McBride and Robertson 1963; Jones 1969*a*, 1969*b*) is likely to be less important in this situation, as the inbreeding effect of artificial selection has been shown to be dependent on selection intensity and heritability of the selected character (Robertson 1961).

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This paper reports a study on the effect of varying intensities of artificial and natural selection in some of the abovementioned lines after more than 57 generations of selection, in terms of effective population size, rate of inbreeding and the spread of genes (James and McBride 1958; James 1962*a*), as an endeavour to better understand the nature of the selective forces operating in these lines.

Materials and Methods

Experimental

Four large selection lines of the six described in a previous paper (Yoo 1980) were chosen for the present experiment. They are designated as Ua, Ub, CRa and CCa according to the population structure treatments imposed in the first 17 generations. However, as they may be regarded as replicates (Yoo 1980), these designations are immaterial in the present context except for providing cross-reference to earlier publications.

The other two lines could not be maintained by single-pair matings as required for pedigreeing, and so were excluded from this study. These lines had been selected continuously for increased bristle number on one abdominal sternite, the fourth in males and the fifth in females; in each generation 50 pairs of parents were selected at an intensity of 20%. The same character was considered in this study.

To vary the intensity of artificial and natural selection, the following sublines of each selection line were derived equally from the same 50 full-sib families (initial families) in generation (G) 58 (for CRa and CCa) or G69 (for Ua and Ub), and pedigreed for nine generations using single-pair matings. (As two lines only could be handled at a time, G58 and G69, called initial generations here, were chosen arbitrarily as representing a late stage in long-term selection):

- SO Subline*: Fifty pairs with highest scores were selected as parents out of 250 contributed as equally as possible by 50 single-pair matings set up in the preceding generation, that is, selection without replacements for failed matings.
- SW Subline*: Selection was identical to that in SO subline, except failed matings were replaced with reserve matings set up with the next highest pairs, to obtain 250 pairs keeping the contribution of a particular mating at a maximum of 10 individuals.
- RO Subline*: Fifty pairs of parents were taken at random as equally as possible from 50 single-pair matings set up each generation. This allowed 'natural' selection among, as well as within, families.
- RW Subline*: Selection was relaxed as in RO subline, but failed matings were each replaced with another mating from the same full-sib families, that is, 'natural' selection operated only within families.

Sublines were specified after line designations, e.g. Ua-SO. Selection was continued in SO and SW sublines for a further three generations after the pedigree phase, but adopting the procedures used prior to the present experiment. RO and RW sublines of Ua and Ub were also continued for three more generations by 50 random pairs mated in groups of 10.

Single-pair matings were made in vials (2.5 by 7.6 cm) and group matings in cream jars (142 ml). Other culture conditions were described by Yoo (1980).

Analytical

To evaluate various factors affecting effective population size, variance effective population sizes (Kimura and Crow 1963) expected at different stages of a generation were computed, assuming a fixed number of individuals to be randomly selected as parents at each stage: when matings were set up [$N_{e(m)}$]; when failed (mainly sterile) matings were determined [$N_{e(st)}$]; when the progeny were scored [$N_{e(sc)}$]; and when selection was finished [$N_{e(s)}$]. The expected variance of number of offspring selected was based on James' (1962*b*) formulae.

In addition, effective population sizes predicted on the assumption that the inbreeding effect of selection was confined to the first generation [$N_{e(1)}$], or accumulated over several generations [$N_{e(4)}$], were calculated using the realized heritability (Robertson 1961); allowances were made for the variation in the number of progeny scored (Crow and Morton 1955).

Inbreeding coefficients (F) were unknown when the above sublimes were derived, as mass mating had been used since G18. They would have been 0.38 and 0.43 by G57 and G68, respectively, assuming an effective population size of 60 (Yoo 1980). In computing inbreeding coefficients by Cruden's (1949) method, G57 or G68 was regarded as base generation and F was assumed to be zero then, as the rate, rather than the absolute level, of inbreeding is of major interest in this study. 'Realized' inbreeding effective population sizes were then calculated using either the rate of inbreeding estimated by regression of $\log(1-F)$ on generation [$N_{e(F)}$] or final inbreeding coefficients [$N_{e(F)}^*$], following Wright (1931). Also, inbreeding effective population sizes and expected inbreeding coefficients were calculated each generation according to Kimura and Crow (1963).

The proportion of genes in generation t (G_t) contributed by an initial family was computed (James 1972), and its relation to the initial family mean (based on 14 pairs) was investigated. According to James and McBride (1958), the change in proportion of genes after one generation may be measured by the ratio, G_{t+1}/G_t . In the present analysis, $\log [(G_{t+1}/G_t)+1]$ was preferred to the ratio, as the transformation improved the correlation with initial family mean. The transformed values were accumulated over generations excluding the first, and correlation between the sum and the initial family mean was computed. When G_t was zero, the ratio was also assumed to be zero.

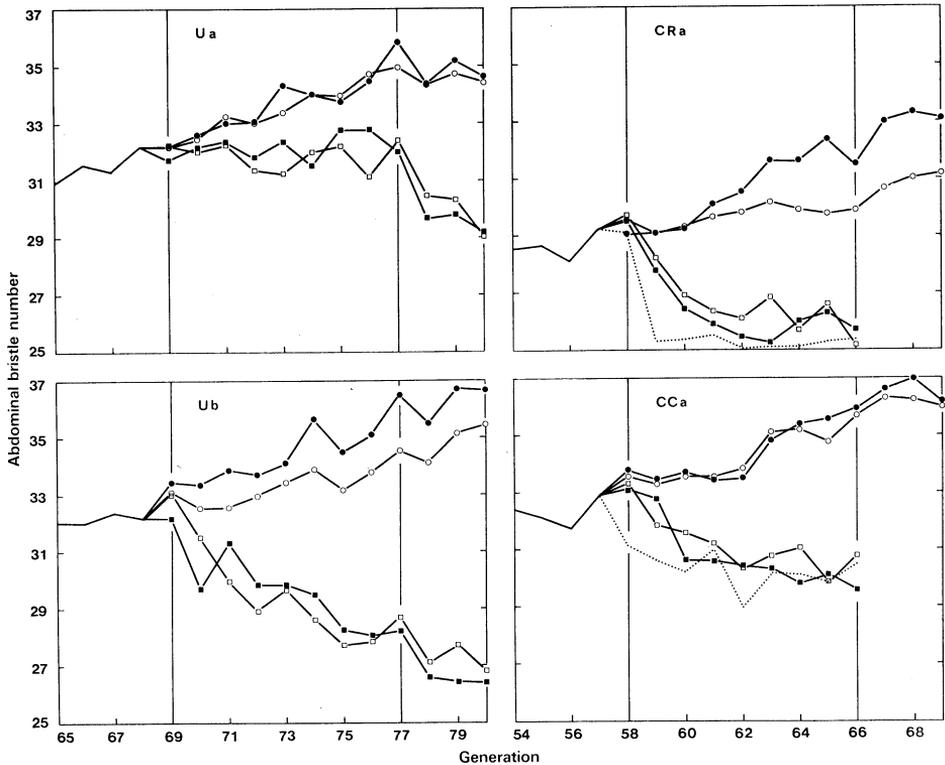


Fig. 1. Mean abdominal bristle numbers in females. Middle sections represent the pedigree period. ● SO subline. ○ SW subline. ■ RO subline. □ RW subline. Crowded relaxed-selection subline.

Results

Response to Selection

In SW sublimes, many reserve matings were recruited each generation, the number being dependent mainly on fertility of a particular subline. So the number of individuals actually selected to be parents was considerably larger than in respective

SO sublines; the harmonic mean numbers were 63.6, 81.4, 65.6 and 57.7 pairs in Ua, Ub, CRa and CCa, respectively.

Table 1. Selection response (*R*), and expected (*ESD*) and realized (*RSD*) selection differential, summed over eight generations, after being standardized by current phenotypic standard deviation and averaged over the sexes

Paired comparison between *ESD* and *RSD* is significant at $P = 0.05$ ($t = 2.79$)

Sublines	<i>R</i>	<i>ESD</i>	<i>RSD</i>	<i>R/RSD</i>
Ua-SO	1.000	11.156	11.187	0.089
Ua-SW	0.745	9.961	9.870	0.076
Ub-SO	0.593	11.249	10.638	0.056
Ub-SW	0.287	8.581	8.391	0.034
CRa-SO	0.591	10.781	10.763	0.055
CRa-SW	0.150	9.577	9.348	0.016
CCa-SO	0.447	10.744	10.519	0.043
CCa-SW	0.605	10.079	9.867	0.061
Average	0.552	10.266	10.073	0.054

Female mean bristle numbers are plotted in Fig. 1. The response pattern was similar in males. Selection response was substantial in all the SO and SW sublines. In Ub and CRa, SW subline lagged behind SO subline, most likely due to the less intense

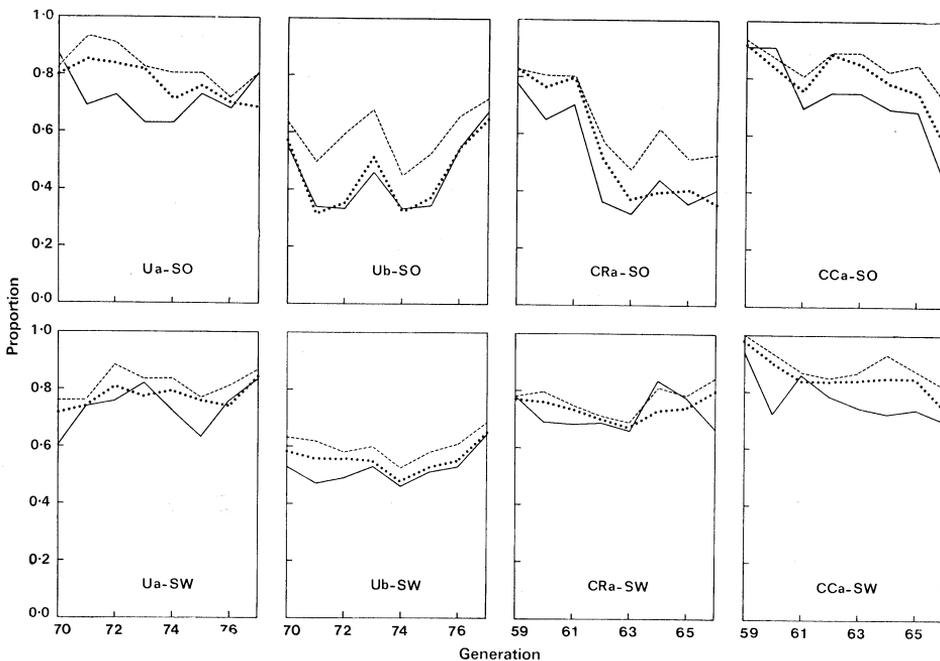


Fig. 2. Variance effective population sizes expected at different stages of a generation as proportions of that expected at mating. --- $N_{e(st)}/N_{e(m)}$ $N_{e(sc)}/N_{e(m)}$. — $N_{e(s)}/N_{e(m)}$. See the text for explanation of the symbols.

selection in the former; and the difference in response remained in the post-pedigree period, although both were subjected to the same selection regime. The difference

between SO and SW sublines was negligible in Ua and CCa. In spite of the efforts to reduce the natural selection, RO and RW sublines declined rapidly in all but one line (Ua). In Ua, RO and RW sublines were remarkably stable during the pedigree period, but started to decline afterwards. Overall, there was little difference between RO and RW sublines.

Fig. 1 shows, in addition to the above sublines, crowded relaxed-selection sublines derived at G58 from CRa and CCA, which have been described earlier (Yoo *et al.* 1980). The crowded culture condition, induced by mating 100 pairs of random parents in one cream jar, was an attempt to increase the intensity of natural selection. The crowded relaxed-selection sublines initially declined more rapidly than respective RO and RW sublines, but the final level was not much different.

The realized heritability as a ratio of total response to total realized selection differential (R/RSD in Table 1), was generally low at this stage of selection with an average of 5.4%. These estimates were used in computing expected effective population sizes under selection. The comparison of expected with realized selection differentials (Table 1) indicates that the abdominal bristle number of parents selected was significantly, but not strongly, associated with the number of offspring scored, the difference being on the average 1.9%.

Table 2. Harmonic mean of observed variance effective population sizes ($N_{e(s)}$), and the comparison with the predicted effective size ($N_{e(1)}$) as ratios to that expected with random selection ($N_{e(sc)}$)

Paired comparison between columns 3 and 4 is not significant at $P = 0.05$ ($t = 0.85$)

Sublines	$N_{e(s)}$	$N_{e(s)}/N_{e(sc)}$	$N_{e(1)}/N_{e(sc)}$
Ua-SO	80.78	0.929	0.906
Ua-SW	109.41	0.952	0.939
Ub-SO	49.36	0.983	0.935
Ub-SW	106.55	0.933	0.974
CRa-SO	53.00	0.912	0.930
CRa-SW	110.73	0.966	0.987
CCa-SO	79.48	0.901	0.954
CCa-SW	102.48	0.912	0.948
Average	86.47	0.936	0.947

Variance Effective Population Size

Fig. 2 shows effective population sizes expected at different stages of a generation as proportions of that expected at mating, $N_{e(m)}$, for SO and SW sublines. This allows a comparison of fertility, fecundity (with an upper limit on the number of offspring scored) and artificial selection for their relative importance in determining the observed effective size, $N_{e(s)}$.

Fertility appeared to be the most important factor affecting effective population size, the reduction ranging from about 10% in CCa-SW to about 40% in Ub-SO. In Ub-SO and CRa-SO (after G62) where fertility was very low, fecundity was relatively important. Otherwise, fecundity and artificial selection appeared to be similar in their relative importance. In most cases, the reduction due to natural selection was less in SW than in SO sublines, as it should be, but that due to artificial selection was not very different.

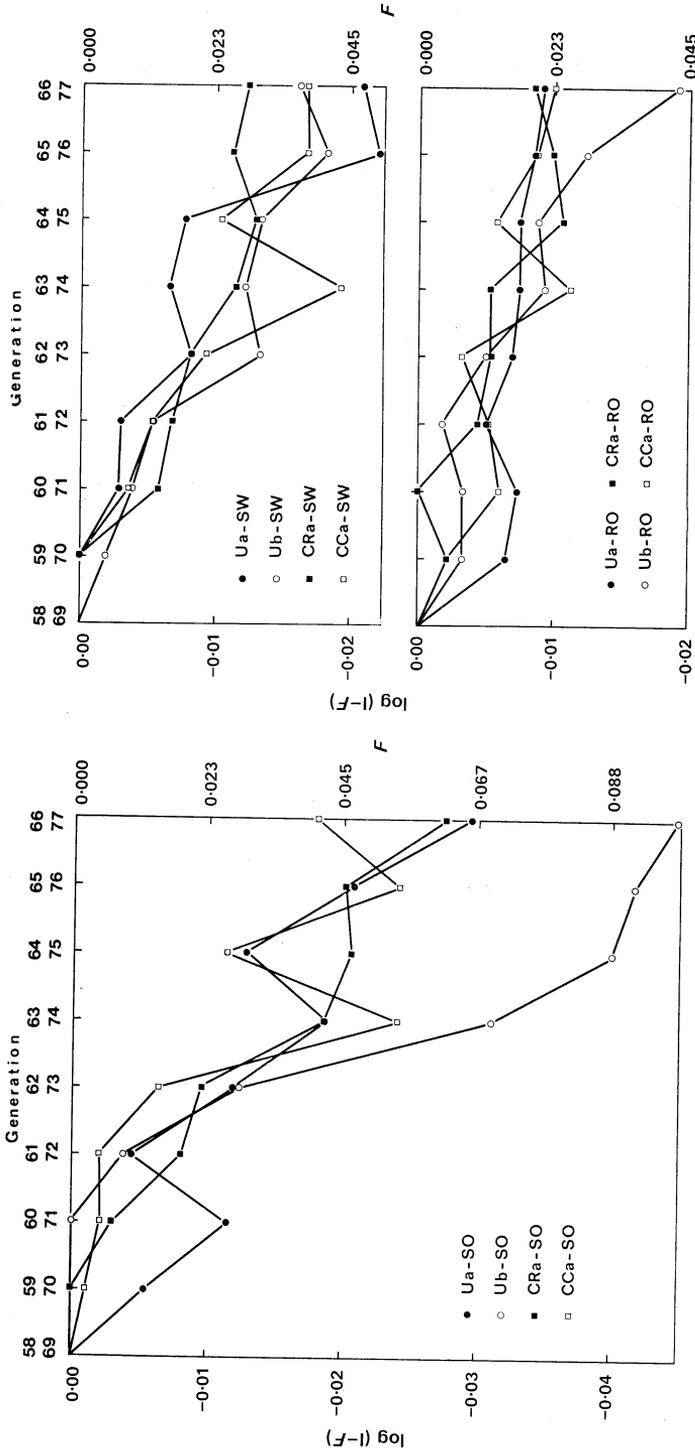


Fig. 3. Mean inbreeding coefficients of parents (F) and $\log(1-F)$ when G57 or G68 was assumed to be the base generation.

Table 2 presents the harmonic mean over generations of observed effective population sizes and its ratio to that expected with random selection. On the average, the immediate reduction in effective population size due to artificial selection was rather small (6.4%) and not significantly different from the prediction (5.3%) based on inbreeding effect of selection in the first generation. However, the agreement between the observed and predicted values was poor for individual sublines, with the correlation coefficient (r) being 0.197.

Table 3. Effective population sizes estimated from the rate of inbreeding ($N_{e(F)}$) and from the final inbreeding coefficient ($N_{e(F)}^*$), and the comparison with the predicted effective size ($N_{e(4)}$) as ratios to that expected with random selection ($N_{e(sc)}$)

Paired comparisons between columns 2 and 3, 4 and 6, and 5 and 6, are not significant at $P = 0.05$ (t values are 2.07, 0.38 and 1.99, respectively)

Sublines	$N_{e(F)}$	$N_{e(F)}^*$	$N_{e(F)}/N_{e(sc)}$	$N_{e(F)}^*/N_{e(sc)}$	$N_{e(4)}/N_{e(sc)}$
Ua-SO	71.96	58.68	0.827	0.674	0.708
Ua-SW	79.50	82.80	0.692	0.721	0.793
Ub-SO	31.40	38.54	0.625	0.767	0.781
Ub-SW	93.40	107.24	0.818	0.939	0.901
CRA-SO	59.92	62.68	1.031	1.079	0.769
CRA-SW	127.71	141.11	1.114	1.231	0.950
CCA-SO	71.44	96.07	0.810	1.089	0.837
CCA-SW	89.75	103.67	0.798	0.922	0.821
Average	78.14	86.35	0.840	0.928	0.820

Inbreeding Coefficient and Inbreeding Effective Population Size

Inbreeding coefficients averaged over selected individuals are given in Fig. 3. RW sublines are not included as their inbreeding coefficients were only slightly smaller than those of RO sublines. The rate of inbreeding was generally low (less than 1% per generation) and $\log(1-F)$ showed non-linear changes over generations in some sublines. Although the mean inbreeding coefficients fluctuated considerably, differences between the sublines were quite clear in the final generation, with SO sublines being around 6%, SW sublines around 4%, and RO and RW sublines around 2%. On the other hand, variation among lines was not large excluding Ub-SO and Ub-RO.

The expected inbreeding coefficient was calculated generation by generation from the inbreeding effective population size. In the final generation, the expected value averaged 4.9% over all SO and SW sublines, very close to the observed (5.2%), and the agreement was good for individual sublines ($r = 0.891$).

The 'realized' inbreeding effective population size was estimated in retrospect from the observed rate of inbreeding, and compared with effective population size predicted from the accumulative effect of selection on inbreeding (Table 3). As would be expected, the inbreeding effect of selection appears more pronounced in these estimates than in the variance effective population sizes (Table 2) except for CRA-SO and CRA-SW, with an average reduction in effective size of 16%. These estimates were not significantly different from and on the average were very close to the predicted, but comparison of individual values showed only fair agreement ($r = 0.431$). When the realized effective population size was estimated from inbreeding coefficient in the final generation, the inbreeding effect of artificial

selection appeared to be smaller, but the agreement between the observed and predicted was much better ($r = 0.712$).

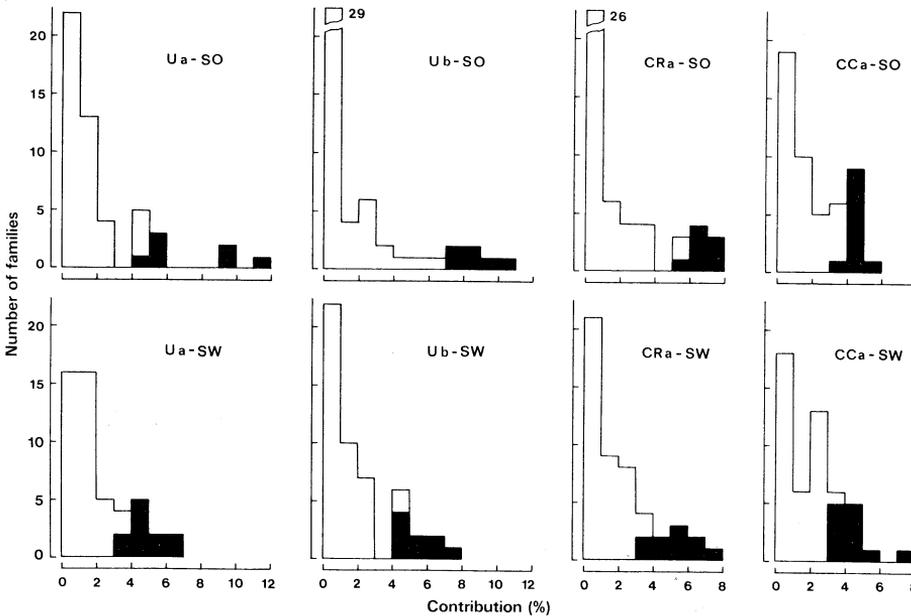
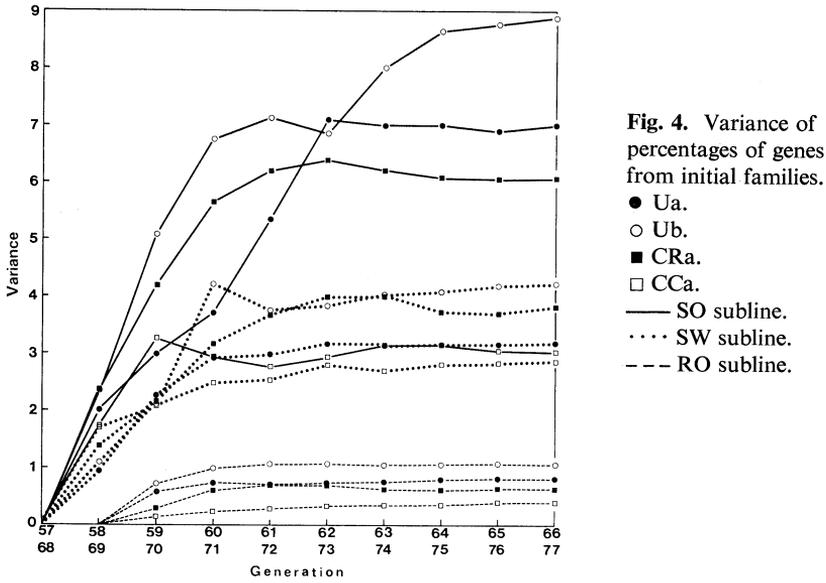


Fig. 5. Frequency distribution of contributions of initial families to the final generation. The families that together contributed about 50% are shown as solid blocks.

Contributions of the Initial Families

The genetic contribution of an initial family is expected to change at a decreasing rate in successive generations. Accumulation of these changes may be expressed in

terms of the variance of percentages of genes contributed by the initial families as shown in Fig. 4 for SO, SW and RO sublimes. In most sublimes, the variance increased asymptotically for the first 4-5 generations to reach a stable level. The spread of genes was much wider in SO sublimes than in SW sublimes, except in CCa, probably because of the less stringent selection in the latter.

The number of initial families represented in the gene pool of a subline decreased mostly in the first three generations, becoming constant by the sixth generation at 28, 31 and 36 in Ub-SO, CRa-SO and Ua-SO, respectively, and at 40 or more in the other selection sublimes. Fig. 5 shows the distribution of gene contributions of initial families to the final generation in each subline. There appeared few outstanding families which made unusually large contributions, and at least six families together made up half the gene pool of any one subline.

The spread of genes in the first generation reflects largely the phenotypic difference between families, but further spread in subsequent generations should be mostly due to additive genetic differences. This genetic influence was measured by correlation between the mean score of an initial family and the accumulated contribution of that family from the second to the final generation (Table 4). The correlations were very low or negative in RO sublimes, but generally high and often significant in SO and SW sublimes except in CRa-SW. These correlations indicate that a measurable quantity of additive genetic variance was still available at this late stage of selection.

Table 4. Correlations between the mean score of initial family and the genetic contribution accumulated over the whole period except the initial generation

* $P < 0.05$. ** $P < 0.01$. † $P < 0.10$

Lines	Subline SO	Subline SW	Subline RO
Ua	0.286*	0.249†	0.045
Ub	0.276*	0.206	0.116
CRa	0.285*	-0.080	-0.069
CCa	0.169	0.514**	-0.218

Discussion

In many long-term selection lines, often characterized by poor reproduction and sluggish or no response, natural selection has been shown to be an important factor opposing artificial selection (for examples, see Al-Murrani 1974). Hence, it is necessary to investigate the nature of natural selection in such populations to understand the forthcoming selection plateaux. Although natural selection was apparently important in the present selection lines (except Ua), as indicated by the rapid regression upon relaxed selection, the following results provide no evidence for direct antagonism between natural and artificial selection: firstly, if the two selective forces operate directly against each other, their simultaneous reduction might not affect the rate of response to selection. In Ub and CRa, where the intensity of selection (artificial and natural) was most different between SO and SW sublimes, SW subline responded considerably less than SO subline, probably due to the less intense artificial selection which was not compensated for by the reduced natural selection. Secondly, the small difference between expected and realized selection differentials suggest lack of strong natural selection associated with bristle number among the selected parents (cf.

Lerner and Dempster 1951). Thirdly, there was no interaction between the two selective forces for the effect on effective population size. Both reduced the effective population size, although the natural selection in this context refers to only that between families. These results contributed to the conclusion (Yoo 1980) that natural selection in these lines was mainly against grossly deleterious genes, particularly lethals, presumably operating through differential pre-imaginal viability, and the opposition to artificial selection was only indirect. Also, this was consistent with the fact that the natural selection in RW sublines was quite effective within families (except in Ua) and the additional between-family selection allowed in RO sublines was not important.

The inbreeding effect of artificial selection is not expected to be great in the present populations in view of the slow response to selection (Robertson 1961). In fact, the average reduction in effective population size was only 6.4% on a single generation basis, a marked contrast with 13.1% estimated in early generations of selection (Rathie 1976). As the inbreeding effect of selection is known to accumulate in successive generations (Robertson 1961), the more appropriate parameter for a selection line seems to be the realized effective population size estimated from observed inbreeding coefficients during the whole period of selection. However, the large sampling variance, and sometimes non-linear change, of inbreeding coefficients make this estimate difficult to obtain with accuracy. This seems to be the reason for the poor agreement between the estimate from the rate of inbreeding based on regression and that from the final inbreeding coefficient. Nevertheless, the correlation with total response (R in Table 1) was -0.466 for the former and -0.812 for the latter, suggesting more inbreeding with greater response. The same conclusion was also reached in short-term as well as in long-term selection with a similar stock of *D. melanogaster* (Jones 1969a, 1969b).

The variance of percentages of genes (Fig. 4) represents the dispersion resulting from natural and artificial selection in successive generations. In SO sublines, where a constant number of individuals were selected, their variances are comparable and may indicate the joint effect of the two selective forces. Therefore, the final level of variance was closely related to $N_{e(F)}^*$, but neither to $N_{e(F)}^*/N_{e(sc)}$ alone, the measure of artificial selection effect, nor to $N_{e(sc)}/N_{e(m)}$ alone, the measure of natural selection effect. That the artificial selection played an important role in the spread of genes, was further confirmed by the moderate and often significant correlations between the mean score of an initial family and its contribution to later generations (Table 4).

The proportion of initial families eliminated during selection was no less in the present SW sublines and Jones' (1969b) previously selected populations than in comparable selection lines at an early stage of selection (Jones 1969a; Rathie 1976), although selection response was much slower in the former. This perhaps suggests that replacing the failed matings was not sufficient to restrain the overriding influence of natural selection in the former, as the replacements were mostly unrelated to the failed matings. Also, the elimination of initial families was much less drastic in all the above populations than in the mass selection lines of McBride and Robertson (1963), perhaps because of the much lower heritability in the former (Jones 1969a).

James and McBride (1958) and McBride and Robertson (1963) observed marked contribution of a few families to later generations, but this tendency was not obvious for the initial families in the present experiment, with the largest contribution of a family being 12% in Ua-SO. The large number of parents and low heritability seem to have prevented rapid concentration of a few families.

It may be summed up that in the selection lines analysed here, natural selection clearly showed the characteristics of selection against grossly deleterious genes, particularly lethals, and artificial selection still capitalized on additive genetic variance with its inbreeding effect being much reduced.

Acknowledgments

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