

Long-term Selection for a Quantitative Character in Large Replicate Populations of *Drosophila melanogaster*

Part 4: Relaxed and Reverse Selection

B.H. Yoo, F.W. Nicholas and K.A. Rathie

Department of Animal Husbandry, University of Sydney, N.S.W. (Australia)

Summary. Reverse and relaxed selection were carried out in sublines which were derived from six replicate lines of *Drosophila* during 86-89 generations of selection for increased abdominal bristle number, and the reverse selection sublines were reciprocally crossed with selection lines of their origin.

The results of serial relaxed selection initiated at different generations of selection confirm that the accelerated responses observed in the selection lines were largely due to deleterious genes, particularly lethals, with large effects on the selected character. The decline in mean bristle number under relaxed selection was not much different between crowded and uncrowded relaxed sublines.

Reverse selection initiated at generation 57 was very effective, though it failed to bring the mean back to the base population level, and the genetic differences between replicate sublines (two from each of the six lines) indicate that low bristle number genes were probably rare in the selection lines. The genes which were still segregating after 57 generations of selection, on the average, did not show any directional dominance. The contribution of the X-chromosome to selection response was proportional to its chromosome length.

Key words: Long-term selection – Relaxed selection – Reverse selection – Dominance of bristle number genes – *Drosophila melanogaster*

Introduction

Reverse and relaxed selection have been used to investigate the strength of natural selection opposing artificial selection and the nature of residual genetic variability of forward selection lines, particularly in laboratory experiments on long-term responses and limits to selection (Robertson 1955; Clayton and Robertson 1957; Roberts 1966; Frankham, Jones and Barker 1968). Recently,

Nicholas (1976) studied consequences of reverse selection with an additive genetic model in the absence of natural selection to provide a theoretical basis of quantitative prediction. Yoo (1980a) reported the results of long-term response to selection for increased abdominal bristle number in six replicate lines of *Drosophila*. The accelerated responses observed in these lines were mostly associated with appearance of non-fixable genes, particularly lethals, with large effects on the selected character. It was argued that these lethals had attenuated artificial selection pressure at later stages of selection, resulting in less genetic gains than would be expected from the residual genetic variability. The analysis of residual genetic variability in these lines (Yoo 1980b) indicated that the additive genetic component was hardly diminished in some of the lines and dominance of high bristle number genes was not an important cause of selection limits (c.f. Falconer 1971).

We report here that the results of reverse and relaxed selection in various sublines split off the six selection lines and of subsequent crossing between these lines and their reverse selection sublines, consolidate the conclusions drawn in the preceding papers.

Materials and Methods

The six replicate selection lines (designated as Ua, Ub, CRa, CRb, CCa and CCb) from which the sublines were split, each consisted of 50 pairs of parents selected upwards at an intensity of 20% (Yoo 1980a). The character scored in the sublines was the same as the selected character, namely bristle number on one abdominal sternite, the fourth in males and the fifth in females. The selection was continued for 86-89 generations (G), during which relaxed-selection sublines were initiated with 25 (up to G25) or 50 pairs taken at random from each line, at intervals of 6 generations up to G49, and in G64 (G65 for some lines) and G82. These sublines had been maintained, each in one cream jar (142 ml), under crowded conditions for 5 (up to G25) or 6 generations before 50 (up to G25) or 100 pairs were scored from uncrowded cultures set up with 10 pairs of parents per cream jar.

At G57 two replicates of each of the following sublines were derived from all lines: (1) Reverse-selection sublines maintained for at least 15 generations, each with 10 pairs selected downward out of 50 scored; the two replicates are denoted by L1 and L2 suffixed to the line designations (e.g. Ua-L1). (2) Uncrowded relaxed-selection sublines each with 10 pairs of parents per generation; 50 pairs were scored from G58 to G65, and in G71. (3) Crowded relaxed-selection sublines continued for 15 generations by 100 pairs of parents in one cream jar; 50 pairs were scored from uncrowded cultures set up separately in each generation.

After 14 generations of reverse selection and then 6 of relaxation, each reverse-selection subline was reciprocally crossed with the main line of its origin. In Ub-L1, in which suspension of reverse selection increased the bristle number, the subline selected continuously was used instead. In addition, reciprocal matings were made between the two replicate reverse-selection sublines from Ub, CRb and CCb. Fifty pairs of progeny were scored from each mating. The degree of directional dominance (D) was estimated from female mean scores as the difference between average of reciprocal crosses and the lower parent relative to the difference between the two parents. The proportionate contribution of the X-chromosome (X) was estimated from male mean scores as the ratio of the difference between the reciprocal crosses to that between the two parents.

Results

Serially relaxed sublines

Figure 1 shows changes in mean bristle number (averaged over sexes) after 6 or 7 generations of relaxed selection initiated at different generations of forward selection. Also included in the figure are the results of the final relaxation of selection in the main lines between G86 and G89; the uncrowded condition used in this case would not bias the comparison between generations, as the effect of crowding was negligible at this stage of selection. That selection was relaxed for one generation more in those sublines derived after G25 would be of little consequence, as most of the change in mean occurred in the first few generations of relaxation.

Relaxed selection resulted in relatively small declines in mean bristle number until G19, but the decline upon relaxation increased a great deal in subsequent generations in all but one line (Ua), mostly after accelerated responses in the main lines, which in turn were associated with occurrence of non-fixable genes, particularly lethals, with large effects on abdominal bristle number (Yoo 1980a). In many cases, the increased decline matched the corresponding accelerated response in terms of the magnitude.

Sublines derived in G57

Figure presents the pattern of change in female mean bristle number for the crowded and uncrowded relaxed-selection sublines derived in G57. As the two replicates in

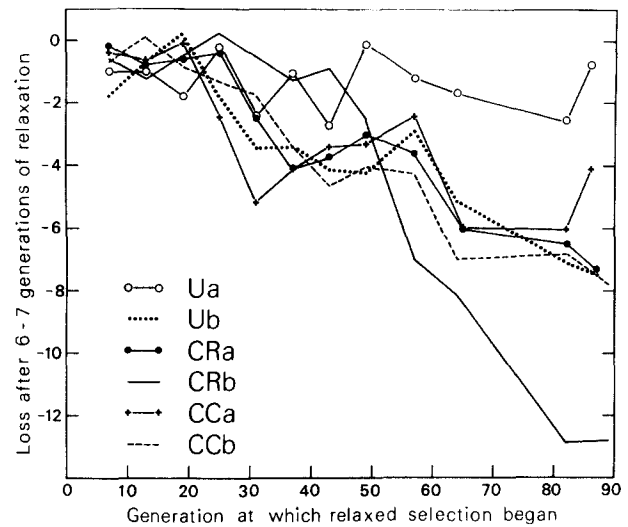


Fig. 1. Loss in mean abdominal bristle number after 6-7 generations of relaxed selection (sexes averaged) plotted against the generation at which relaxed selection began

each case showed much the same trend over generations, only the average has been plotted.

Most of the decline in mean bristle number occurred in the first 2-5 generations in all relaxed sublines, although those from Ua and CCa showed some stepwise changes under crowded conditions. The crowded culture condition, which was imposed as a means of intensifying 'natural' selection, had no consistent effect on the rate of decline or on the level of downward plateau reached.

The pattern of response to reverse-selection in females is also depicted in Figure 2. In general, mean bristle number declined more rapidly and reached lower levels in reverse-selection than in relaxed-selection sublines. In Ua, CRa and CCa, the two replicate sublines behaved similarly, but in the rest, the agreement between replicates was very poor, with one replicate in each case showing a clear accelerated response in later generations similar in size to the initial decline. Ub-L1 and CRb-L2 reached an apparent limit only 3 and 5 bristles above the base population mean, respectively, while CCb-L2 and Ub-L2 plateaued at higher levels. The remaining 8 sublines appeared to be still responding to selection slowly when the reverse selection ceased, probably approaching plateaux well above the base level.

Selection was relaxed in additional sublines taken from all reverse-selection sublines in G65. In Ub-L1, Ub-L2 and CRb-L2, where reverse-selection was continued longer, relaxed sublines were again derived in G72; at the same time, reverse-selection was finally relaxed in the others. Overall, mean bristle number changed very little upon relaxation of reverse-selection except in Ub-L1, and thus only a part of the results is shown in Figure 2. In Ub-L1,

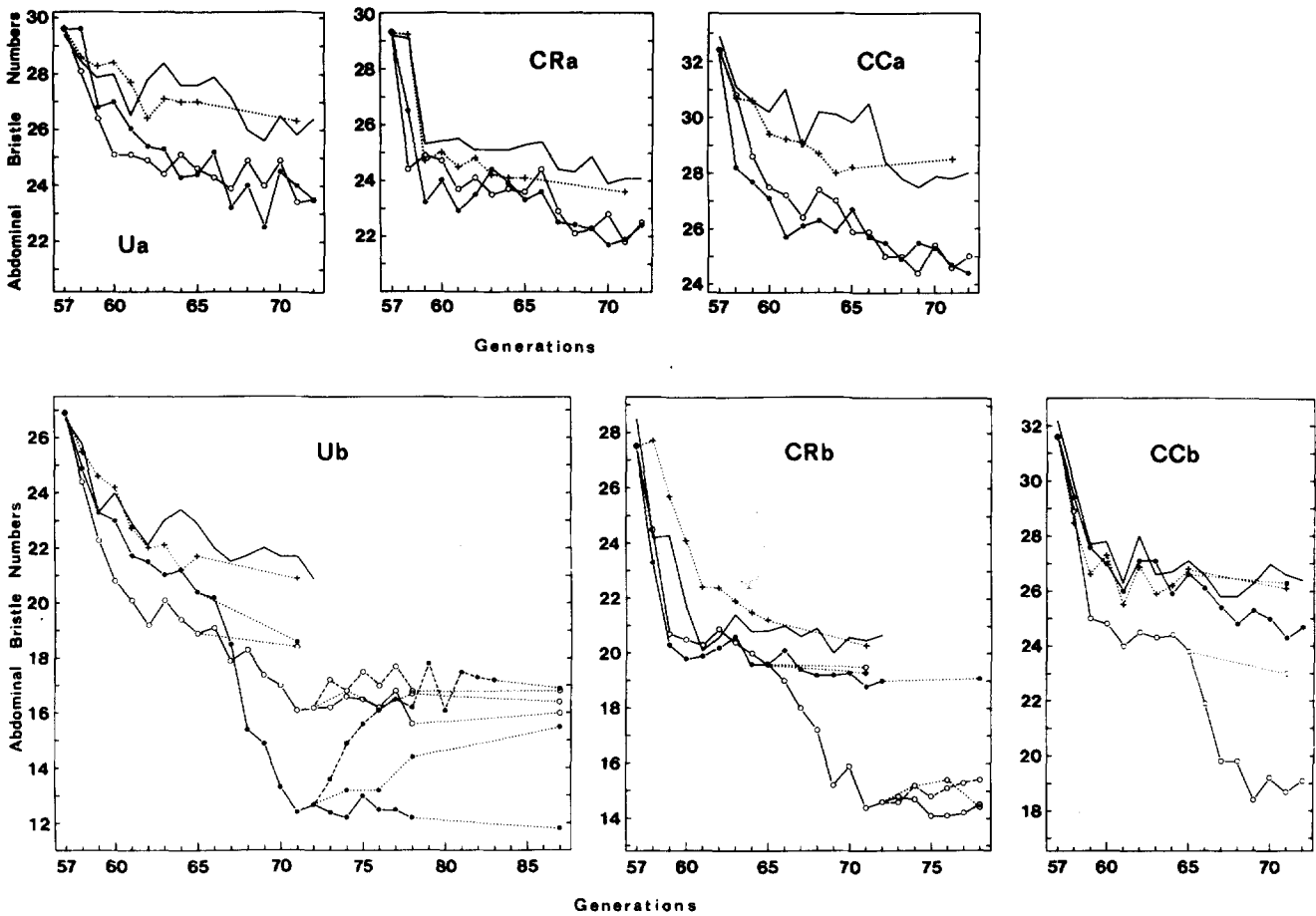


Fig. 2 Female generation means of crowded (continuous line) and uncrowded (cross) relaxed-selection sublines averaged over two replicates, and of two replicate reserve-selection sublines L1 (full circle) and L2 (open circle). For reserve-selection sublines, responses to 'switch-back' (broken line) and to relaxed (dotted line) selection are also shown.

Ub-L2 and CRb-L2, 'switch-back' selection sublines, where reverse-selection was reversed again, were derived in G72, but selection response was appreciable only in Ub-L1.

The reverse-selection sublines of Ub were unique in that (1) relaxed selection, except that at G78, always moved the population mean towards seemingly an equilibrium level of about 16.5 bristles, (2) the 'switch-back' selection in Ub-L1 showed an immediate response to reach a limit at the same level, and (3) fertility was very poor in Ub-L1 around G72, but improved considerably when reverse-selection was suspended or switched back.

The changes in generation mean were proportionately similar in the two sexes. However, female means only were plotted in Figure 2, as they sometimes show the pattern of change, particularly accelerated responses, more clearly.

Directional dominance and X-chromosome contribution

D and X values were estimated from the cross between each main line and reverse-selection subline (Table 1). In crosses between Ub and its sublines, D values were significantly larger than 0.5, i.e. the genes increasing bristle number on average tended to dominance over their alleles. The opposite tendency was observed in CRb. In the remaining lines, D values did not deviate significantly from 0.5. Hence, the mean over all lines (0.51) was very close to 0.5 expected for additive genes.

Also presented in Table 1 are similar estimates from crosses between the two replicates of reverse-selection subline derived from Ub, CRb and CCb. Note that a gene or genes differing between Ub-L1 and Ub-L2 showed probably complete dominance.

Table 1. The degree of directional dominance (D) and the proportionate contribution of X-chromosome (X) estimated from crosses between main selection lines and their reverse-selection sublines and between the two replicates of reverse-selection sublines

Parental lines or sublines ^a	D	X
Ua Ua-L1	0.502 ± 0.055	0.267 ± 0.083 ^b
Ua Ua-L2	0.580 ± 0.055	-0.045 ± 0.081
Ub Ub-L1	0.645 ± 0.037 ^b	0.645 ± 0.038 ^b
Ub Ub-L2	0.592 ± 0.039 ^b	0.139 ± 0.045 ^b
CRa CRa-L1	0.538 ± 0.074	0.414 ± 0.100 ^b
CRa CRa-L2	0.445 ± 0.062	0.045 ± 0.080
CRb CRb-L1	0.384 ± 0.031 ^b	0.086 ± 0.048
CRb CRb-L2	0.443 ± 0.029 ^b	0.020 ± 0.040
CCa CCa-L1	0.496 ± 0.039	0.236 ± 0.071 ^b
CCa CCa-L2	0.484 ± 0.045	0.017 ± 0.072
CCb CCb-L1	0.576 ± 0.095	-0.400 ± 0.131 ^b
CCb CCb-L2	0.471 ± 0.041	0.095 ± 0.053
Ub-L2 Ub-L1	1.132 ± 0.181 ^b	1.287 ± 0.154 ^b
CRb-L1 CRb-L2	0.788 ± 0.216	-1.652 ± 1.785
CCb-L1 CCb-L2	0.443 ± 0.071	0.262 ± 0.097 ^b

^a Higher lines or sublines given first

^b Significantly different from 0.5 (D) or from zero (X) at P < 0.05

The X values show that the contribution of the X-chromosome was about two thirds of the difference between Ub and Ub-L1, but only 14% of that between Ub and Ub-L2. This may be fully accounted for by the difference between Ub-L1 and Ub-L2 being entirely due to the X-chromosome. A sex-linked genetic factor exhibiting complete recessiveness seems to have been responsible for the additional decline in Ub-L1 below Ub-L2.

Although the response to reverse-selection was very similar between replicates in Ua, CRa and CCa, the genetic changes underlying the phenotypic similarity were quite different in that the X-chromosome made significant contributions only in one replicate from each line. It is hard to explain that the X-chromosome of CCb-L1, in spite of the downward selection, increased abdominal bristle number relative to that of CCb. The mean of absolute values of X over all lines was 0.20, indicating that the contribution of the X-chromosome was approximately proportional to its chromosome length.

Discussion

The decline in mean bristle number under relaxed selection increased irregularly at later stages of selection in all lines but Ua, largely reflecting accelerated responses observed earlier in the main lines. This supports the contention that the accelerated responses were mainly due to deleterious genes which had large effects on abdominal

bristle number or were closely linked to other genes with such effects (Yoo 1980a). Further, the comparison of crowded with uncrowded relaxed-selection sublines indicates that the natural selection operating under these conditions was 'hard' selection independent of competition level (Wallace 1968), consistent with the involvement of lethals and other deleterious genes of similar nature. Replicate variation of reverse-selection response was large in Ub, CRb and CCb. In the other lines, in spite of the similarity of response, the two replicate sublines differed significantly in contribution of the X-chromosome, implying that the genetic basis underlying the response was not the same. The number of relevant loci still segregating after 57 generations of selection would have been limited, with the genes for high abdominal bristle number being fairly common. In other words, low bristle number genes were probably rare when reverse-selection was started, resulting in high replicate variation due to the founder effect (James 1971). It seems reasonable to explain the delayed, rapid response in some reverse-selection sublines by crossovers between closely linked genes. In the case of Ub-L1, the recombinant appeared to be recessive and sex-linked.

As directional selection is not effective in eliminating recessive genes, some undesirable recessives, if present, are likely to persist even after long-continued selection (Falconer 1971; Al-Murrani and Roberts 1974). In the present selection lines, however, there was no strong tendency to directional dominance for those genes segregating at G57. Since undesirable dominant genes would have been mostly eliminated by then, this implies that dominance was not an important feature of the base population, as was shown by statistical analyses (Hammond 1973). The lack of directional dominance in this study contrasts with the slight recessiveness of high bristle number alleles (D = 0.426) in essentially the same base population (Frankham 1974). One explanation for the difference could be that the lethals with large effects on bristle number, present in the main lines, tended to move the F₁ mean closer to the mid-parental mean, although the D value was not lower in the line without such lethals (Ua).

Table 2. The comparison of forward- and reverse-selection responses (averaged over lines, replicates and sexes)

	Mean ± S.E. ^a
Forward response to G 57 (Δx)	17.85 ± 0.79
Reverse response from G 57 to G 72 (R)	8.07 ± 1.01
R/ Δx	0.46 ± 0.07
Decline under relaxed selection (L)	4.40 ± 0.62
(R - L) / (Δx - L)	0.29 ± 0.07

^a Standard errors were calculated from the variance among the six lines

According to Nicholas (1976), the ratio of reverse-selection response to preceding forward-selection response would be 0.193 in this experiment, assuming that the effective population size was 60 during selection in either direction and that the 'proportionate effect' of a locus (Falconer 1960) was not greater than 0.012. A more general form of this original formula, which allows for differences in effective population size between forward and reverse-selection, predicts a ratio of 0.153. The observed ratio (Table 2) was considerably higher than the predicted, suggesting that the forward-selection was less effective and/or the reverse-selection was more effective than expected because of the natural selection, probably through the lethals mentioned above (Yoo 1980a).

Acknowledgements

This work was done while B.H.Y. held a University of Sydney Research Studentship. We are grateful to Professor J.S.F. Barker for his encouragement and advice. The technical assistance of Patricia Brown and Nanette Hardy is appreciated.

Literature

- Al-Murrani, W.K.; Roberts, R.C. (1974): Genetic variation in a line of mice selected to its limit for high body weight. *Anim. Prod.* 19, 273-289
- Clayton, G.A.; Robertson, A. (1957): An experimental check on quantitative genetical theory. 2: The long-term effects of selection. *J. Genet.* 55, 152-170
- Falconer, D.S. (1960): Introduction to quantitative genetics. Edinburgh: Oliver and Boyd
- Falconer, D.S. (1971): Improvement of litter size in a strain of mice at a selection limit. *Genet. Res.* 17, 215-235
- Frankham, R. (1974): The nature of quantitative genetic variation in *Drosophila*. 2: Average dominance of abdominal bristle polygenes. *Austr. J. Biol. Sci.* 27, 683-686
- Frankham, R.; Jones, L.P.; Barker, J.S.F. (1968): The effects of population size and selection intensity in selection for a quantitative character in *Drosophila*. 3: Analyses of the lines. *Genet. Res.* 12, 267-283
- Hammond, K. (1973): Population size, selection response and variation in quantitative inheritance. Ph.D. Thesis, University of Sydney
- James, J.W. (1971): The founder effect and response to artificial selection. *Genet. Res.* 16, 241-250
- Nicholas, F.W. (1976): Forward and reverse response to artificial selection. *Theor. Appl. Genet.* 48, 101-104
- Roberts, R.C. (1966): The limits to artificial selection for body weight in the mouse. *Genet. Res.* 8, 361-375
- Robertson, F.W. (1955): Selection response and the properties of genetic variation. *Cold Spring Harb. Symp. Quant. Biol.* 20, 166-177
- Wallace, B. (1968): Topics in population genetics. New York: Norton
- Yoo, B.H. (1980a): Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. 1: Response to selection. *Genet. Res.*
- Yoo, B.H. (1980b): Long-term selection for a quantitative character in large replicate populations of *Drosophila melanogaster*. 3: The nature of residual genetic variability. *Theor. Appl. Genet.*

Received September 29, 1979

Accepted November 6, 1979

Communicated by J.S.F. Barker

B.H. Yoo

Division of Animal Production, CSIRO

P.O. Box 184 North Ryde, N.S.W., 2113 (Australia)

F.W. Nicholas

Department of Animal Husbandry

University of Sydney

N.S.W., 2006 (Australia)

K.A. Rathie

Dairy Cattle Husbandry Branch

Department of Primary Industry

Newstead, Queensland, 4006 (Australia)