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Asymmetrical Responses to Automatic Selection for Body Size in *Drosophila melanogaster*

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Summary. An apparatus has been made for the automatic selection of *Drosophila* for body size, operating on the principle of a fractionating sieve. The measurements of individual flies by this method were approximately normally distributed and the repeatability of measurements on successive days was 0.5. A two-way selection experiment for this character was carried out with two replicates for ten generations. The realised heritability for the measured score was 0.14 ± 0.02 for high score and 0.20 ± 0.02 when it was for low score. The correlated response in body weight was asymmetrical, the change downwards being much greater than that upwards. There was a clear divergence in activity measurements between the lines selected in the two directions but no clear trends in fertility. Examination of the selected lines after eleven generations showed that the relationship between score and body weight was clearly different in the lines selected in the two directions and was non-linear in both.

It is suggested that the response in activity observed as a consequence of selection for score is partly due to the direct response for activity and partly to a correlated response because of a negative genetic correlation between body size and activity. The observed non-linear relationship between score and body weight observed within generations may be a direct cause of the asymmetry of direct and correlated responses which may also have a parallel in other situations.

Introduction

The great majority of selection experiments with *Drosophila* have involved bristle counts, either on the abdomen or on the sternopleurum. The labour involved is considerable, particularly for those lines selected upwards. For this reason there have been several attempts to find traits which could be selected for automatically, in particular behavioural traits. Hirsch and Tryon (1956) devised an apparatus for the automatic measurement of geo- and phototaxis in *Drosophila.* Lerner and Inouye (1968) selected *Tribolium* for maze running performance and their method would also be applicable to *Drosophila.* Grant and Mettler (1969) worked on escape behaviour in *Drosophila.* All these characters have the advantage that very large numbers of flies can be measured. In the case of geotaxis, however, there is the disadvantage of an absolute limit to selection response. In general these methods have the disadvantage of being very sensitive to environmental variation and therefore being extremely difficult to standardise for use in different laboratories as well as having a low heritability.

Although many selection experiments have been carried out on body size in *Drosophila,* in particular the series by Reeve and F.W. Robertson (1952), these have all involved individual measurement. In this paper we present an automatic method of selection of *Drosophila* for body size based on ability to pass through a series of graded slits. We have used the method to select replicate lines with a control population. The automatic method has the attraction that a large number of individuals can be run at the same time so that intense selection can be carried out without causing a high rate of inbreeding.

Material and Methods

The selection procedure was based on the principle of the fractionating sieve. It proved technically difficult to construct graded meshes of sufficient accuracy in the necessary range of 0.8 mm to 1.3 mm and we therefore used a single slit, the width of which could be calibrated accurately. The apparatus consisted of a linear series of compartments with a calibrated slit in the separating walls. The width of the slit between compartments declined gradually over the whole apparatus. This was made from transparent material so that the flies could be watched undisturbed. It was mounted in a dark room and a light beam shone through all the compartments along its length. With increasing distance from the source, the slits connecting neighbouring compartments became wider. At the end away from the light source, a starting vial could be inserted containing flies to be measured. In spite of the attraction by light, not all the flies were willing to pass through the compartments and so, to encourage them, escape behaviour was induced by putting small cotton wool tips soaked with 25 % acetic acid into each compartment. They were left there during the whole run producing a repellent atmosphere so that the flies try to escape towards the light, being forced to squeeze themselves through as many slits as they possibly could or would. The total run lasted one hour - we ob-

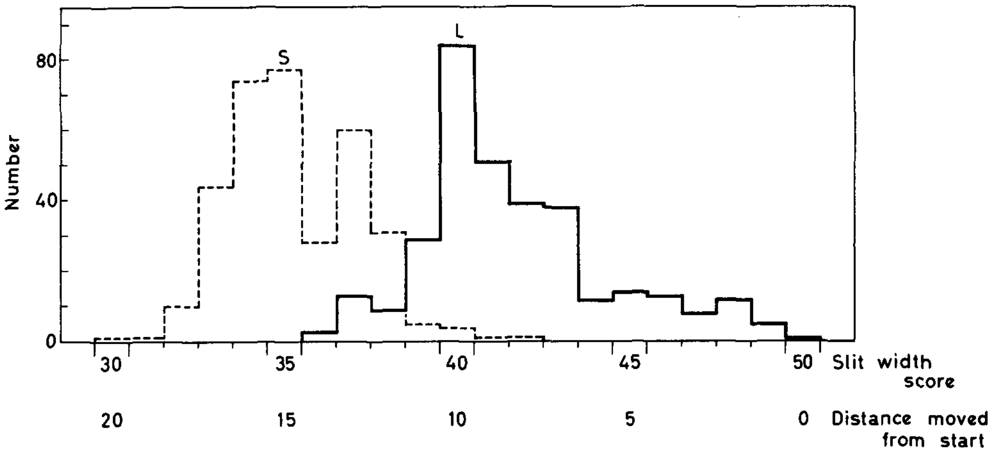

Fig.1. The distribution of score in a high and in a low replicate at generation 11

served that after this time the distribution of flies over the compartments had become stable. The base material for the selection experiment was a population derived from a recent capture at Riudevella in Spain. We selected two replicates in each direction, using as a control a white-eyed stock which could easily be distinguished from the flies in the selection lines. We were thus able to measure both control and selected flies automatically and the same time. The flies selected for breeding in each line were mated in a separate bottle but freshly laid eggs of selected and the corresponding control lines were placed in the same culture. In both selection and control lines, 40 males and 100 females were used as parents each generation. Selection was carried out only in males, the 40 extreme males being selected from 300 in the apparatus. 100 white-eyed control flies were measured at the same time.

Three other measurements were made each generation - body weight, motor activity and fertility. For the measurement of activity an apparatus was used similar to the automatic selection apparatus in all respects but with a constant width of slit, which was large between all compartments. The running time was about 25 minutes and the flies had to escape from the acetic acid by moving towards the light source. Because the slits were now large in relation to body size they could be traversed freely. Fertility was measured as the number of adult progeny produced under conditions of low larval competition by 100 females laying eggs over a period of 15 hours.

Results

The distribution for two of the selected lines at generation 12 gives an indication of the degree of separation both within and between lines produced by the automatic fly scorer. The flies are put in at the right hand side of the apparatus, where the slits are widest and the light source shines from the left. For clarification two scales are given on Fig.1, one in terms of slit width and the other in terms of the number of cells moved through. If the main interest is in body size of the flies, a convenient scale is given by the width of the slit in the compartment in which the fly is found at the end of the run. On the other hand if the flies are assessed for their motor ability, the appropriate scale would be reversed. Since our main objective was to measure the body size we shall make use of the slit width scale and this character will be called the 'score'. It must be remembered therefore, that high scores mean, on one hand, large body size but on the other that few compartments have been passed through from the beginning of the run. The distribution of scores are satisfyingly normal, although with 300 flies a smoother distribution would be expected. This lack of smoothness comes from unequal increases in slit-width from compartment to compartment due to difficulties of calibration. The calibration had been made under a microscope using a micrometer eyepiece. This gave a series of slits differing in width between adjacent slits by 0.027 mm but it did so on average only.

Fig.2 shows the generation means for the four traits measured for the two replicates of two-way selection. The direct response given at the top of the figure appears to be symmetrical. However, because the phenotypic variance was larger in the lines selected for high body size there was a difference in the realised heritability in the two directions.

The realised heritability for positive selection for score was 0.143 ± 0.016 and for negative selection 0.204 ± 0.018, with standard errors computed by the method of Hill (1972). There is a significant difference between the two estimates. The correlated responses in body weight are clearly asymmetrical, the

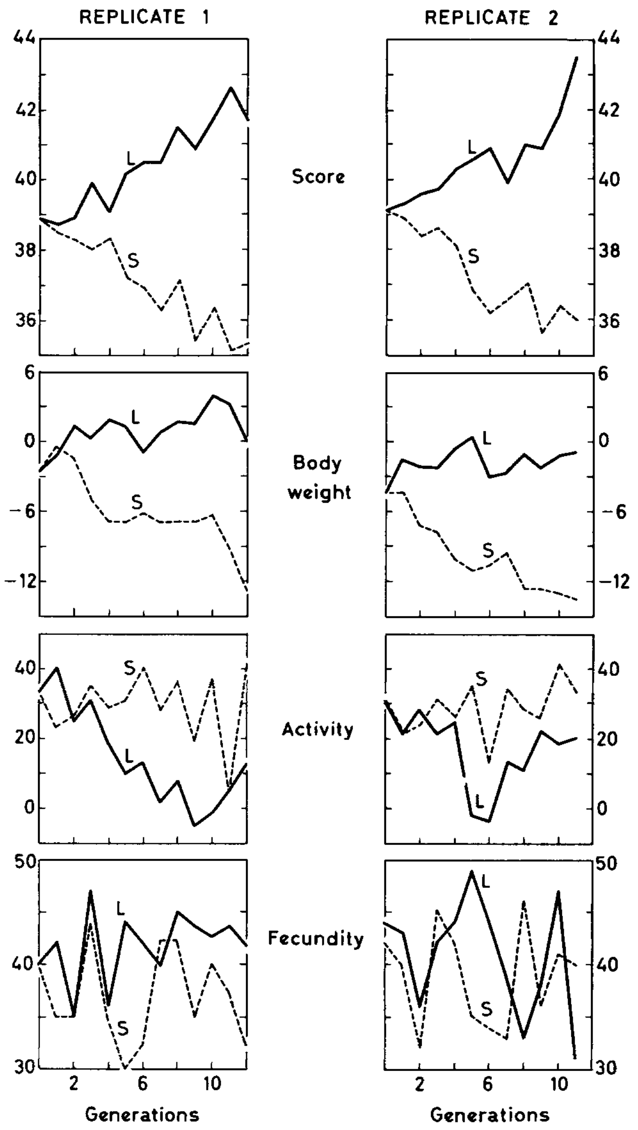

Fig.2. Direct response in score and correlated responses in body weight (in gms · 10^{-5}), activity (as the deviation from controls in the percentage of flies above an arbitrary score in an apparatus with wide slits) and fertility (as relative numbers of progeny produced by a single female in uncrowded conditions)

change downwards being much greater than that upwards in both replicates. The response in activity was also asymmetrical, the line selected for low score altering little with a clear decline in activity in the line selected for high score. There was no clear trend in the correlated response for fertility - the large flies having a higher fertility in the first replicate but not in the second.

Observation of the flies during runs in the automatic fly scorer gave a subjective impression of considerable differences within runs between the behaviour of those flies which had moved little and those which had moved a great deal. The former appeared to be less insistent in trying to squeeze through the slits than the latter. To look further into this we made a more detailed analysis at the 11th generation of selection. One high and one low replicate was taken and 1600 newly emerged males were taken from each. In each line the flies were separated into 4 groups and run in the fly scorer. The flies in the different score classes were then weighed, with the results given in Fig.3. The relationship between score and body weight is clearly curvilinear. There must be flies which stay close to their starting point at score 50 even though their body size would allow them to go further. The separation effected by the apparatus in body size is clearly different in the two lines, being much better for the low line than for the high line. After weighing the flies, the extreme scoring groups were run for a second time in the automatic apparatus to estimate repeatability. The regression of score in the second run on that in the first, which was used to estimate the repeatability of the character, proved to be 0.41 ± 0.05 for the high line and 0.75 ± .05 for the low line, the difference between the two values being significant.

Discussion

The nature of the inter-relationship between activity and body size is critical for the interpretation of these results. Even if there were no genetic correlation between the two, it is clear that in this apparatus selection operates simultaneously for both characteristics. On the flies found in the high scoring section, a higher proportion will be there through inactivity than will be found in the low scoring section. But possible pleiotropic relationships between activity and body size further complicate matters. We are fortunate that Dr. van Dijke of the University of Utrecht has recently selected *Drosophila* from a similar strain to ours for their activity in a similar apparatus but with wide apertures between chambers. He has produced two strains very different in activity, which he made available to us. The flies of the active strain, when reared together with those of the passive strain under good conditions, weigh some 20 % less than the latter. Dr. van Dijke kindly ran our strains in his apparatus and confirmed the difference in activity between them which was, however, much less than that between his

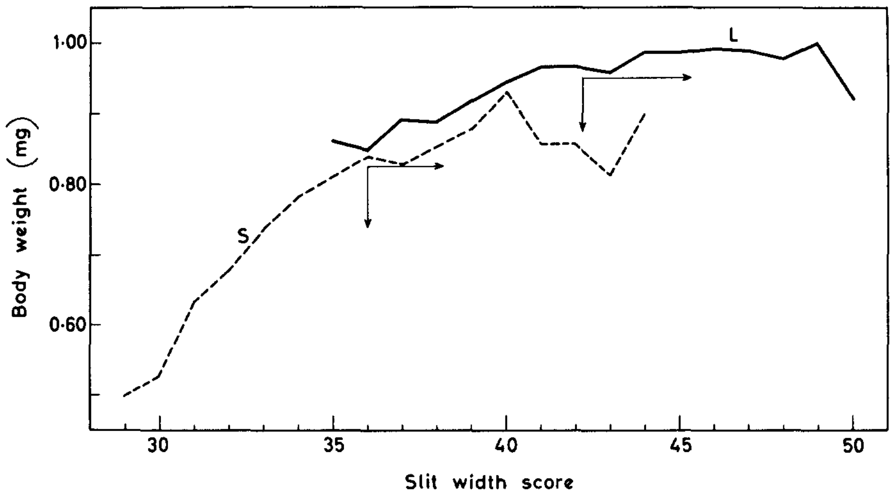

Fig.3. Phenotypic regression of body weight on score at generation 11. Arrows indicate the means and standard deviations

strains. It therefore seems clear that there is a negative genetic correlation between body size and activity and that the response in activity in our strains is partly direct and partly correlated. But we must remember that activity itself may be complex. Ewing (1963) selected *Drosophila* for reduced spontaneous activity and concluded that he had in fact selected not for activity but for a claustrophobic behaviour pattern which prevented the flies from entering narrow passages connecting the compartments of his activity maze. In our experiments we may therefore have been selecting not only for exploring behaviour but also the willingness of the fly to squeeze through slits.

To look further into the consequences of an interrelationship between body size and activity, we considered as a possible model of the situation the mathematical relationship between score (S), weight (w) and activity (a) $S = 10 \tan^{-1}(w/a)$.

The factor 10 is arbitrary and serves as a convenient transformation of scale. This relationship has the desired property that the importance of body weight in determining the score depends on the mean activity in the individuals concerned. If the mean activity is high, score is mostly determined by weight and if the activity is low the score is mostly determined by activity. Selection for score, based on such a relationship between weight and activity, was then simulated on a computer. Normal genotypic and environmental components were simulated for the two component characters, assuming a genetic correlation of -0.5 and a phenotypic relationship of zero. Both characters were assumed to have a phenotypic standard deviation of unity and heritabilities of 0.4 and 0.15 for body weight and activity respectively. Selection for score was then simulated for 10 generations with initial population means of five and three units respectively. The results for selection in the two directions were quite clear in that there was not only a clear asymmetry of response in score but also, in a more pronounced way, in body weight.

These results then indicate that asymmetry of selection response may result when a composite trait is based on a few component characters which interact in a non-linear manner. The importance of this will depend on the extent of variation in the two characters. In the present instance, the non-linear effects operate within the range of variation of a single generation to produce a non-linear regression of body weight on score. It is then not surprising that non-linear effects are also observed when selection responses over several generations are considered. As selection proceeds the interaction between the components may well be altered. An example of this comes from the work of Land and Falconer (1969), selecting for spontaneous ovulation rates in the mouse. Selection for high ovulation rate produced a response due to an increase in FSH-activity whereas the response downwards was due to an increased sensitivity of the ovaries to the hormone. This then provides an alternative kind of explanation for the asymmetry of direct and correlated responses to that put forward by Bohren et al. (1966) which is in terms of pleiotropic effects contributing in the opposite direction to the genetic covariance between the two characters. This may be a more general phenomenon than has previously been realised.

Acknowledgement

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