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Genetic analysis of larval feeding behaviour in *Drosophila melanogaster*

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SUMMARY

The larvae of *Drosophila melanogaster* feed continuously during their period of development. The rate of feeding activity, measured as the number of cephalopharyngeal retractions per minute, varies with the physiological age of the larva. Feeding rate responded readily to directional selection to give rise to non-overlapping populations with fast and slow feeding larvae, respectively. Realized heritabilities for the character from different selected lines varied between 11 and 21 %. Crosses between the selected populations show significant dominance for fast feeding rate and appreciable non-allelic gene interaction. Larvae of the slow feeding populations showed a correlated reduction in locomotor activity but fast feeding larvae do not move about significantly faster than the unselected controls. Asymmetry of the correlated response to selection, it is argued, is due to selection in the slow feeding populations of alleles with a secondary effect in both behaviours.

1. INTRODUCTION

Although the behaviour of adult *D. melanogaster* has been the subject of extensive investigation (see Parsons, 1973), little is known about larval behaviour, despite its possible importance in the development of the organism. In feeding bottles larvae spend much of their development feeding on the substrate, and this constitutes a major behavioural character in the larval life-cycle. The feeding action consists of successive extensions and retractions of the mouth hooks actuated by the cephalopharyngeal sclerites accompanied by pumping action of the pharynx.

The rate of larval feeding may be expected to have effects on the rate of larval development. Bakker (1969) selected larvae differing in speed of development, and concluded that early or late pupation time was dependent on the rate of feeding. Kearsey (1965) reached a similar conclusion in an investigation concerning the interaction of competition and food supply in *D. melanogaster*. Bakker (1961) suggested that differences in competitive ability between a wild-type strain and a *Bar* mutant strain were due to small differences in feeding rate, and that such a difference between groups might be important when they live together on a limited food supply. Bakker argued that rapid feeders would consume more food than slow feeders, and that this might have significant effects when the slow feeders failed to attain the minimum weights necessary for pupation and emergence as a

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viable adult. Thus, differences in larval feeding behaviour may influence larval growth and survival, and so affect the structure of the gene pool of the population. Unfortunately, neither Bakker nor Kearsey made direct behavioural observations on feeding rate, and consequently their conclusions are necessarily speculative. The study reported here was designed to investigate whether changes in the rate of larval feeding activity can be produced in response to selection, and how this character may be related to other components of larval and adult behaviour.

2. MATERIALS AND METHODS

The base population on which selection was applied was established by means of reciprocal crosses between Amherst, Florida, Formosa and Samarkand inbred lines of *D. melanogaster*, maintained in the Department of Genetics at Sheffield University. This foundation stock was kept as a mass-bred stock throughout the study on a live yeast–agar–oatmeal–molasses medium at 25 ± 1 °C, on a light–dark cycle of 14 h light and 10 h darkness.

Adults from the selected lines were allowed to lay eggs on to agar-coated watch-glasses for a 4 h period. On hatching, larvae were transferred to agar-filled watch-glasses, coated with a thick layer of fresh yeast paste, and allowed to develop. Fifty larvae were plated on to each watch glass to avoid crowding effects. It is important that larval density should be carefully controlled because culture density significantly affects the subsequent performance of single larvae in the feeding rate test. On the third morning following hatching, when the larvae were approximately 70 h old, their feeding rates were measured. This was accomplished by transferring single individuals from their rearing environment to a Petri dish containing 3 % agar, on which a 2 % yeast suspension had been poured to a depth sufficient to allow larvae to move freely. Yeast suspension was used to provide a source of food for the larvae, and also to enable continuous observation of each individual. (When larvae are observed *in situ* they tend to burrow into the substrate and pass out of view.) Each larva was allowed 1·5 min to recover from the transfer, and was then observed for a test period of 1 min of continuous feeding, during which time a feeding activity score was obtained by counting the number of cephalopharyngeal retractions. After being scored the larvae were assigned to groups with a range of scores covering 5 units. When measurement was complete larvae were transferred to appropriately marked 4 × 1 inch feeding vials and left to develop. Approximately 30 larvae were placed in each vial, and on emergence the adults were segregated and kept until 3–4 days old, at which point they were brought together for mating and egg-laying.

Replicates of each experimental and control line were maintained, and in each generation 120 individuals from each selection line and 30 from each control line were measured. Selection pressure was maintained by selecting as parents for the next generation approximately the top or bottom quartiles of the fast and slow feeding lines, respectively. The control lines were maintained on a random breeding schedule, and reserve stocks of all lines were available throughout the experiment.

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For observations on larval feeding rate in aseptic synthetic medium eggs were harvested from each of the selected lines, sterilized following the procedure described by Sang (1956), and germ-free larvae inoculated on to a modified version of Sang's medium-C (Sang & Burnet, 1968). Their feeding rate was scored whilst larvae were feeding *in situ* in the medium, by observing them through the walls of the glass culture tube.

For measurements of locomotor activity, larvae were removed from their rearing environment and placed on 3% agar, where they were left to move about on the agar substrate. This served both to familiarize them to the environmental change and also their activity removed any yeast adhering to the larval body. Individual larvae were then placed on another Petri dish containing 3% agar and allowed 1·5 min to recover from the transfer. At the end of this period the test period of 1 min commenced. Larval locomotor activity was recorded by counting the number of forward or reverse movements made by the larva during the test period. All measurements were made in the morning on mid-third instar larvae.

Adult locomotor activity was measured in an open field apparatus made from a white perspex box 10 × 10 × 0·5 cm with a transparent lid marked off in centimetre squares. The procedure is described in detail by Connolly (1966). A single fly was introduced into the apparatus and allowed a recovery period after transfer of 1·5 min. The number of lines crossed in one minute was then recorded. Observations were made using 3 to 4-day-old virgin adult flies, between 9 a.m. and 12 noon to avoid diurnal rhythm effects.

All behavioural observations were made at 25 °C in the same constant-temperature room in which the larvae and adult flies were cultured.

3. RESULTS

(i) *Feeding and locomotor activity of larvae*

Under optimum culture conditions larvae feed continuously, on or in the substrate, persistently working the medium with their mouth hooks. Apart from breaks at the ecdysis feeding continues throughout the period of larval development. Under crowded conditions breaks in feeding activity frequently occur spontaneously, when larvae come into contact. Feeding activity in single larvae placed in a drop of yeast suspension generally proceeds without breaks, and the rate is independent of the concentration of yeast over a range of 0·25–25%. Fig. 1 shows that feeding rate, measured by the rate of cephalopharyngeal retraction varies with the physiological age of the larvae. The rate tends typically to increase during the first and second larval stadia, remains relatively stable during the first half of the third instar and then drops towards the time of pupation (Fig. 1).

Larvae *in situ* move about as they feed, crawling over the substrate or tunnelling into it. Their locomotor activity can be measured in an open field situation by transferring them individually on to a plain agar substrate without food. In this situation the larvae crawl about actively. The crawling movements involve forward extension of the head accompanied by stabbing of the mouth hooks into the sub-

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strate for leverage. A sequential wave of extension followed by retraction of each segment in turn passes down the body posteriorly and when it reaches the anal end the head is again extended and the cycle repeated. A track of paired mouth hook prints is left in the agar surface as the larvae progress. The rate of larval locomotion remains relatively constant across the larval period as illustrated in Fig. 1.

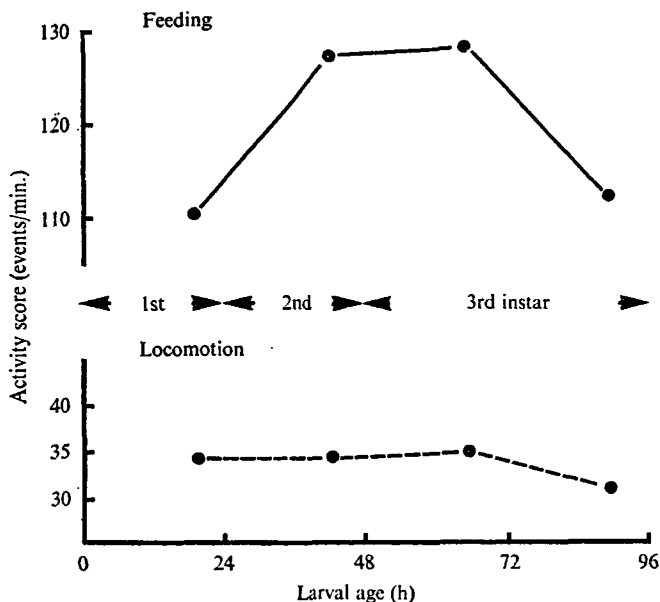

Fig. 1. Rates of feeding and locomotor activity in larvae of the base population, related to physiological age.

(ii) *Selection for change in larval feeding rate*

Selection for larval feeding rate was made using larvae aged 70 h after eclosion as described in Materials and Methods. Two replicate lines FA and FB respectively, were selected for fast feeding rate. Two replicate lines SA and SB, respectively, were selected for slow feeding rate, and two control lines CA and CB were measured in each generation. The mean feeding rate in each of these lines remained stable between 140 and 150 retractions per minute throughout the experiment. Fig. 2 shows the response plotted against the applied selection differential. In the fast lines there was a steady response to selection up to a plateau in the region of 60 retractions/min above control in FA, and 50 retractions/min above control in FB, respectively. Selection for slow feeding rate was also effective. In the SA line there was no further response to selection beyond a mean value of approximately 45 retractions/min below control. Feeding rate in the SB line reached a lower level to fluctuate around a mean in the region of 65 retractions/min below control. The total range between the fast and slow lines after the selection limit had been reached was $6{\cdot}7\sigma_p$ and $7{\cdot}3\sigma_p$ for the A and B lines respectively, where σ_p is phenotypic standard deviation for the base population.

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Realized heritability estimates based on the linear portion of the response illustrated in Fig. 2 are given in Table 1. The heritability for rate of larval feeding activity for this population is low in comparison to that for adult locomotor activity ($h^2 = 0{\cdot}51 \pm 0{\cdot}10$) observed by Connolly (1966) using a mass bred Pacific population of the same species.

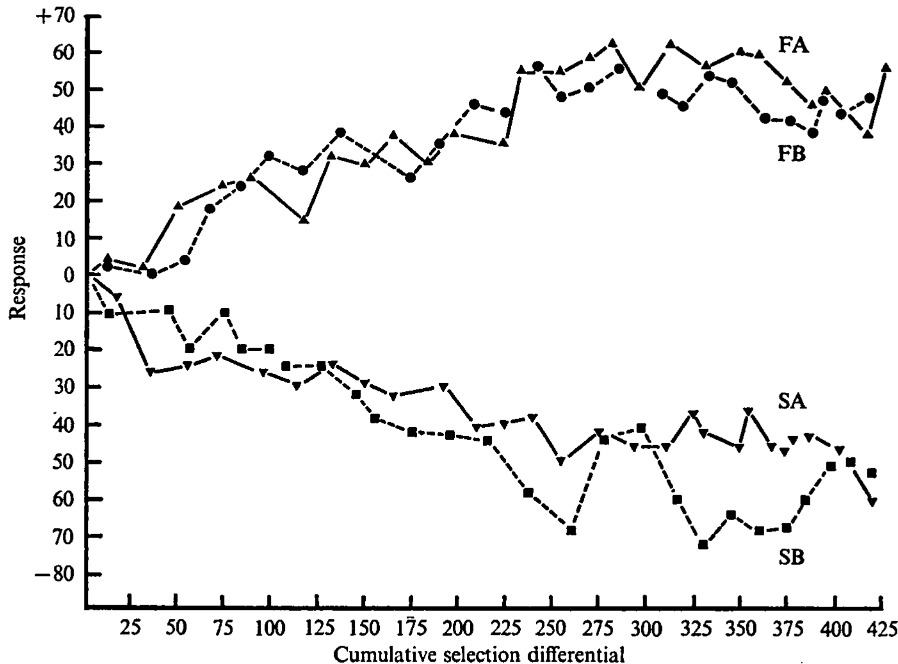

Fig. 2. The relation between cumulative selection differential and the response to selection, expressed as the deviation from the control unselected population, in lines selected for rate of larval feeding activity at 70 h after emergence from the egg.

Table 1. *Realized heritability for larval feeding rate*

(Values of $h^2 \pm$ s.e. have in each case been computed from the regression of response (up to F16) against selection differential illustrated in Fig. 2.)

	Fast	Slow
Series A	$0{\cdot}205 \pm 0{\cdot}018$	$0{\cdot}197 \pm 0{\cdot}022$
Series B	$0{\cdot}112 \pm 0{\cdot}014$	$0{\cdot}209 \pm 0{\cdot}023$

The distributions of phenotype scores illustrated in Fig. 3 show substantial separation between the fast and slow lines by generation 6. By generation 12 the tails of the distributions of fast and slow lines of the A series just overlap whereas in the B series they are quite separate. Beyond the point at which selection progress has reached a standstill the fast and slow lines of both series show discrete non-overlapping distributions of phenotype scores. The mean larval feeding rate remained stable for several generations of relaxed selection in lines derived from

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the plateaued FA, FB and SA populations, but there was significant regression towards the control value with five generations of relaxed selection in the SB line, indicating that genetic heterogeneity still remained in this population after the selection limit had been reached.

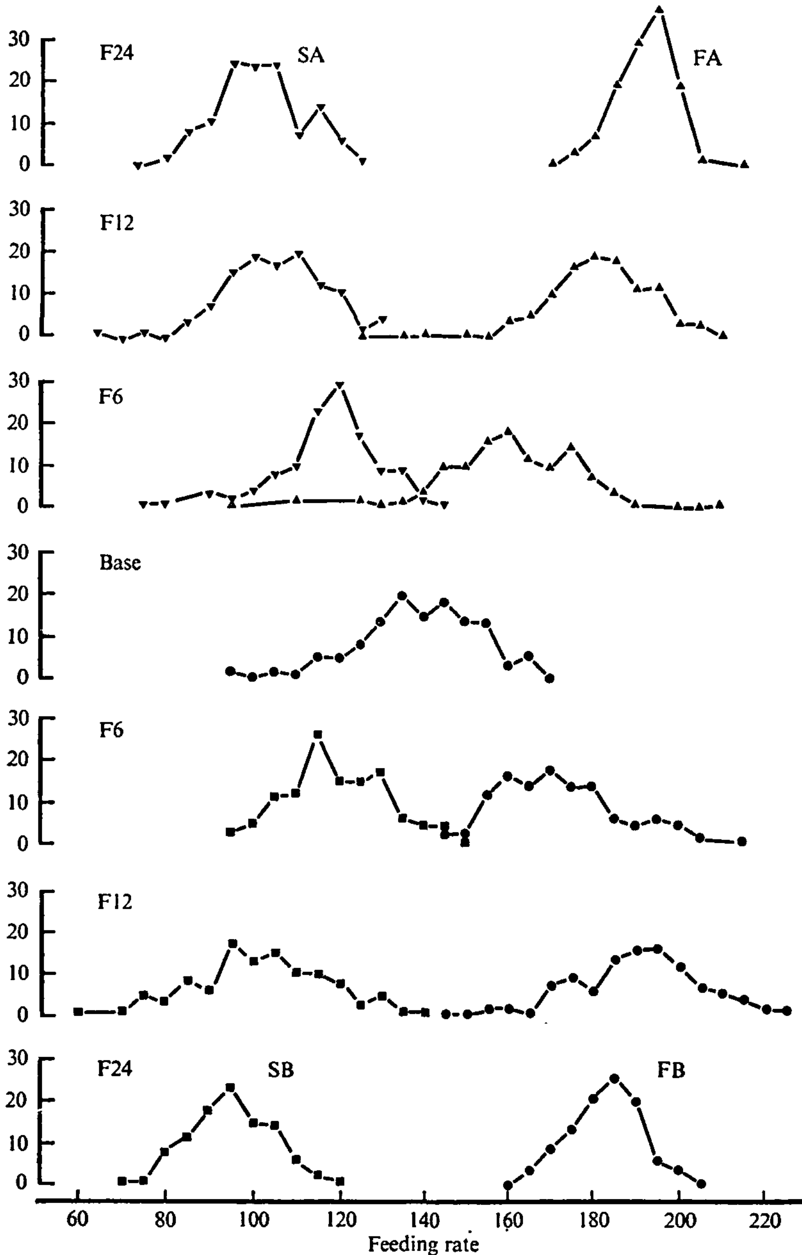

Fig. 3. Distribution of phenotype scores in the base population and in the selected lines.

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(iii) *Stability of selection gains in different environments*

Larvae were scored for feeding rate in a 2% yeast suspension and selection made for the character as expressed under these conditions. This raises the question of whether the gains under selection may be maintained in an environment closer to that of the semi-solid food medium normally provided under standard culture conditions. The agar–oatmeal–molasses–yeast medium routinely used for culture media is too opaque to permit observation of larvae feeding *in situ*, but the translucent agar gel used for synthetic media overcomes this problem (see Materials and Methods).

Table 2. *Mean feeding rate for 70 h old larvae of selected lines FA and SA measured at 25 °C on a 2% yeast solution, and for germ-free larvae feeding* in situ *on a modified version of Sang's medium C*

	Fast	Slow
Yeast suspension	194·4 ± 1·14	104·3 ± 1·52
Aseptic medium	206·5 ± 1·95	130·5 ± 2·51

The data summarized in Table 2 show that under germ-free conditions on the synthetic agar gel medium a difference between selected lines is maintained but is smaller than on yeast suspension. The mean rate in both stocks is higher on the synthetic medium but there is evidence of genotype–environment interaction in that the proportional increase in feeding rate is greater in the slow than in the fast line. Changes in feeding activity in response to selection are evidently not specific to larval behaviour in 2% aqueous yeast suspension and represent real differences in feeding activity between the selected populations on more conventional gel media. The increase in the rate of feeding activity in larvae raised on synthetic medium compared with their performance on live-yeast medium seems to indicate that some component of Sang's medium C is normally limiting for feeding rate on live-yeast.

(iv) *Correlated responses in larval and adult locomotor activity*

Although selection pressure was applied only to the rate of larval feeding activity it is possible that systematic changes in other behavioural characters correlated with feeding activity might occur during the course of the experiment. Such a correlated response could arise in a variety of different ways. Genes controlling the rate of larval feeding activity may have pleiotropic effects on other measures of behaviour. There may for example be a 'general activity factor' present in the organism which controls the rates of activity of otherwise discrete behaviours. A transient correlation between measures might also be caused by linkage between genes controlling the rates of activity in the different behaviours. Knowledge of the underlying cause of the phenotypic correlation between measures would not of course allow us to predict the direction of the relationship which could be positive, or negative, in each case.

Feeding and locomotor activity in larvae are at least superficially similar in

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that both involve the use of the mouth hook apparatus. Larvae of the base population used for selection showed a positive but non-significant correlation ($r = +0.19$) between these measures. The correlated response in larval locomotor activity in each of the lines selected for larval feeding activity, measured in each case as the deviation from the intra-generation control, is shown in Fig. 4. In both slow lines

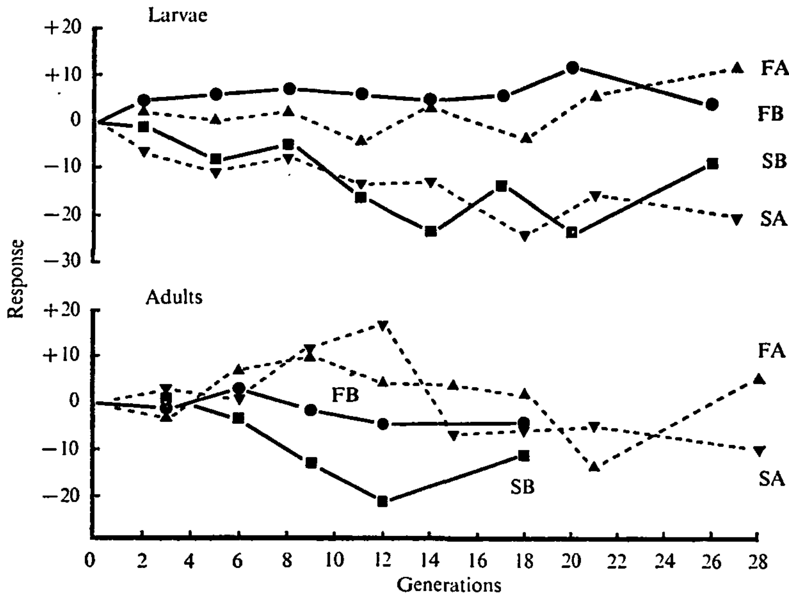

Fig. 4. Correlated responses in locomotor activity of adult flies and of larvae in each of the lines selected for rate of larval feeding activity. Rates of locomotor activity are in each case expressed as the deviation from the intra-generation unselected control population.

Table 3. *Feeding rate and locomotor activity in the lines selected for high (active) and low (inactive) adult locomotor activity*

(The score for each behaviour is expressed as the mean followed by its standard error.)

	Active	Inactive
Adults		
Locomotor activity	57.5 ± 1.30	28.1 ± 1.95
Larvae		
Feeding rate	122.6 ± 1.83	141.6 ± 2.38
Locomotor activity	43.4 ± 1.29	47.1 ± 1.10

(SA and SB) there was a consistent and significant reduction in larval locomotor activity ($P < 0.01$ in each case). There was no significant trend in locomotor activity in the fast feeding line (FB), but in the FA line an increase in locomotor activity occurred between generations 18 and 27 ($P < 0.01$).

Locomotor activity in the adult flies developing from the larvae of the selected lines showed no directional trend accompanying change in the rate of larval feeding

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activity (Fig. 4). There seems to be no indication of a phenotypic correlation between these measures. Further evidence relevant to this relationship is given by the data, summarized in Table 3, for two populations selected for differences in adult locomotor activity (Connolly, 1966). These lines differ significantly in the rate of adult locomotor activity and in the rate of larval feeding activity. Feeding rate is fastest in the line with low adult locomotor activity and vice versa, and the rather small difference in larval locomotion between strains is also in the same direction as the difference in feeding rate.

(v) *Genetic analysis of selected lines*

The mean phenotype scores for the selected lines FA and SA, after the selection limit had been reached, and for the F_1, F_2, and each respective backcross were computed from the raw data scores. In addition, the observations were examined using a series of alternative transformations of scale and analysed using the scaling tests described by Mather (1949). Homogeneity of variance in the non-segregating populations P_1, P_2 and F_1 was most nearly approached by using the untransformed scores. Additivity was achieved by none of the transformations used.

Table 4. *Mean and variance for larval feeding in the selected lines FA and SA (after F25) and in the* F_1, F_2 *and backcross generations*

					Backcross	
	Fast	Slow	F_1	F_2	$F_1 \times F$	$F_1 \times S$
n	100	60	80	120	120	120
Mean	198·2	102·0	175·8	171·3	182·0	112·6
Variance	107·0	152·8	129·2	191·1	147·7	225·9

The results shown in Table 4 for the untransformed scores indicate dominance for fast feeding rate (which was still present after logarithmic and other transformations were used) and, as would be expected, the variance for the backcross to the recessive (Slow) parental strain is greater than the variance for the backcross to the dominant (Fast) parental strain. The results therefore indicate dominance and non-allelic interaction in the control of larval feeding rate.

4. DISCUSSION

The rate of feeding activity in larvae responded readily to bidirectional selection to give non-overlapping populations with fast and slow feeding individuals, respectively. The quite rapid divergence between the FA and SA lines under selection was due largely to the initial response for slow feeding rate, which indicates that some few alleles with relatively major effect were quickly recruited into this line. The two slow lines also differed markedly in their final phenotype scores beyond the selection limit. Another indication that the slow lines differ in their genetic structure is that under relaxed selection the SA line was stable whereas the

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SB line regressed rapidly towards the mean of the control base population, and this may perhaps be due to heterozygosity for alleles determining slow feeding rate which are deleterious when homozygous. The crosses between fast and slow lines FA and FB show departures from additivity, and since these could not be removed by scalar transformation the indications of aggregate dominance for fast feeding rate and appreciable non-allelic interaction must be regarded as real. More extensive and detailed genetic analysis of the selected lines is being undertaken.

Changes in feeding rate may be expected to relate to fitness through effects on the rate of larval development and growth relations affecting body size. A detailed report on these parameters in the selected populations will be given elsewhere.

Drosophila like other holometabolous insects has during its development a succession of contrasting life styles in larva and adult which make it, in a sense, two organisms in one. As Wigglesworth (1954) points out, it is only by virtue of the independence of these successive stages that natural selection may operate in different ways to produce independent evolution of the organism in the two successive stages of its life-history. Locomotor activity in the larva and adult fly are unlikely to constitute homologous behaviours because different morphological structures are involved in their performance and so it seems probable that different genetic systems will be involved in controlling them. This is not to say, however, that genes which affect activity in the adult may not have pleiotropic effects on aspects of larval behaviour. The neurological mutant *Hyperkinetic*, which causes abnormal electrophysiological activity in the thoracic ganglion, affects different measures of adult activity (Kaplan, 1972) and also has pleitropic effects on the rate of larval feeding activity (Burnet, Connolly & Mallinson, 1974). Nevertheless, the absence of significant correlated responses to selection seems to be in accord with the view that activity parameters of larvae and adult flies are independent at the genetic and behavioural levels.

Feeding and locomotion in the Drosophila larva are similar to the extent that both depend on actuation of the cephalopharyngeal sclerites and so presumably these activities are subserved by similar neuromuscular mechanisms. This poses the question of whether there is any relationship which might point to the existence of a common factor determining the levels of activity for these measures within the same phase of development. Such a factor, it has been suggested, may underlie certain aspects of activity in the rat (Lát & Gollová-Hémon, 1969). There was no systematic change in locomotor activity correlated with a quite substantial increase in feeding rate, although a small increase was observed in the FA line after 18 generations of selection. Progress towards slow feeding rate was, however, accompanied by a correlated reduction in locomotor activity in both selected lines. Slow feeders move about more slowly than larvae in the control lines from the base population. The asymmetry of the correlated response to selection suggests that, in addition to an increase in the frequency of alleles with a direct effect in reducing the rate of feeding activity *per se*, alleles at gene loci with a secondary effect on feeding rate may also have been selected. These could influence the rates of otherwise discrete components of behaviour by causing, for example, depression

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of the metabolic rate or some general impairment of the efficiency of the neuromuscular system. In the natural state a high rate of feeding together with rapid mobility in food search may confer advantage in conditions of food shortage, but under optimal environmental conditions an increased propensity for locomotor activity could be maladaptive if it causes reduction in the time spent feeding. Consequently there would be an advantage if these behaviours are regulated independently. The present results indicate that the rates of feeding and locomotor activity are served by different control systems in the larva of Drosophila.

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REFERENCES

BAKKER, K. (1961). An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. *Archives Neerlandaises de Zoologie* **14**, 200–281.

BAKKER, K. (1969). Selection for rate of growth and its influence on competitive ability of larvae of *Drosophila melanogaster*. *Netherlands Journal of Zoology* **19**, 541–595.

BURNET, B., CONNOLLY, K. & MALLINSON, M. (1974). Activity and sexual behavior of neurological mutants of *Drosophila melanogaster*. *Behaviour Genetics* **4**, 223–231.

CONNOLLY, K. (1966). Locomotor activity in *Drosophila*. II. Selection for active and inactive strains. *Animal Behaviour* **14**, 444–449.

KAPLAN, W. D. (1972). Genetic and behavioral studies of *Drosophila* neurological mutants. In *Biology of Behavior* (ed. J. A. Kiger), pp. 133–157. Corvallis: Oregon State University Press.

KEARSEY, M. J. (1965). The interaction of competition and food supply in two lines of *Drosophila melanogaster*. *Heredity* **20**, 169–181.

LÁT, J. & GOLLOVÁ-HÉMON, E. (1969). Permanent effects of nutritional and endocrinological intervention in early ontogeny on the level of non-specific excitability and on lability (emotionality). *Annals of the New York Academy of Sciences* **159**, 710–720.

MATHER, K. (1949). *Biometrical Genetics*. London: Methuen.

PARSONS, P. A. (1973). *Behavioural and Ecological Genetics*. Oxford: Clarendon Press.

SANG, J. H. (1956). The quantitative nutritional requirements of *Drosophila melanogaster*. *Journal of experimental Biology* **33**, 45–72.

SANG, J. H. & BURNET, B. (1968). Physiological genetics of melanotic tumors in *Drosophila melanogaster*. V. Amino acid metabolism and tumor formation in the *tu bw*; *st su-tu* strain. *Genetics* **59**, 211–235.

WIGGLESWORTH, V. B. (1954). *The Physiology of Insect Metamorphosis*. Cambridge University Press.

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