Theoretical and Applied Genetics 43, 291–297 (1973)

Genotype-Environmental Interaction in the Activity and Preening of *Drosophila melanogaster*

DAVID A. HAY

Departments of Genetics and Psychology, University of Birmingham (England)

Summary. Techniques, recently developed to analyze genotype-environmental interaction in plants, are used to study the behaviour of two inbred lines of *Drosophila melanogaster* and their F_1s. The locomotor activity and preening of the hybrids altered far less with age and between two different test conditions than did the behaviour of their homozygous parents. In one apparatus, age affected the additive genetical component leading to heterosis for high activity and, in the other condition, a maternal effect on activity was age-dependent. Preening varied far less with age but, like activity, showed dominance for low inter-individual variability and differences between replicates, due entirely to the unstable performance of one inbred line.

In the light of additional evidence on larval-adult survival and on adult viability, it is suggested that a negative correlation exists between viability and responsiveness to many types of environmental variation. Therefore information may be gained by analyzing inter-individual variability, instead of the customary practice of rescaling data to make the variances homogeneous.

While the locomotor activity and preening behaviour of *Drosophila melanogaster* are subject to genetical control (Hay, 1972a), the expression of this control was found to vary with the rearing conditions and the form of apparatus used for testing. The reason for these fluctuations was not unreliability of measurement, but rather that the inbred lines being tested were far more sensitive to many kinds of environmental change than were the hybrids between them.

Such phenomena are not uncommon in psychogenetics (Roberts, 1967) and differences between genotypes in their environmental responsiveness have been reported for several behavioural traits, e.g., for the effects of temperature on the mating behaviour of *Drosophila* homo- and heterokaryotypes (Parsons and Kaul, 1966). Because estimates of genetical variation may be made unrealistic and even negative through such genotype-environmental interaction, Broadhurst and Jinks (1961) and more recently Newell (1970) advocated rescaling the data to eliminate the effects of this interaction on the variances, rather than trying to estimate its magnitude. Yet these interactions may provide useful information; both Broadhurst and Jinks (1966) and Roberts (1967) suggested that there may be a selective advantage in not responding to environmental changes and there is indeed evidence for a negative correlation of variability with viability in *Drosophila* (Dobzhansky and Levene, 1955; Hay, 1972a).

Using two inbred strains known to differ in viability both at the larval and adult stages, the present paper examines this relationship with behavioural variability in detail, by means of systematic analyses recently developed by plant geneticists to study sensitivity to environmental fluctuations and outlined below.

In the absence of interaction between genes at different loci, the generation means for two inbred lines, $\bar{P}_1$ and $\bar{P}_2$, and their $\bar{F}_1$ can be specified in terms of three parameters only; $\mu = 1/2\,(\bar{P}_1 + \bar{P}_2)$ (the overall mean) and $[d] = 1/2\,(\bar{P}_1 - \bar{P}_2)$ and $[h] = \bar{F}_1 - 1/2\,(\bar{P}_1 + \bar{P}_2)$, which are respectively the additive and dominance genetical contributions to the means summed over all loci irrespective of the degree of association or dispersion of the genes. (See Jinks and Jones (1958) and Mather and Jinks (1971) for fuller details.) But additional parameters are needed when the genotypes react differently to some environmental variable; Bucio Alanis and Hill (1966) showed that the means in any environment j may then be defined as:

$$\bar{P}_{1j} = \mu + [d] + e_j + g_{dj}$$
$$\bar{P}_{2j} = \mu - [d] + e_j - g_{dj}$$
$$\bar{F}_{1j} = \mu + [h] + e_j + g_{hj}$$

where μ, $[d]$ and $[h]$ are calculated as above, except that they are now averaged over all environments. The deviations from these average values of μ, $[d]$ and $[h]$ are specified for the jth environment by the three components:

e_j = additive environmental contribution of the jth environment;

g_{dj} = interaction of the additive genetical component $[d]$, with environment j, that is, g_{dj} is the difference between $[d_j]$ and the overall $[d]$;

g_{hj} = interaction of the dominance component, $[h]$, with environment j.

In many instances, the magnitudes of g_{dj} and g_{hj} have been found to be linearly related to e_j over the whole range of the values of j, that is, over all en-

vironments. The expectations can then be rewritten as

$$\bar{P}_{1j} = \mu + [\bar{d}] + (1 + \beta_d)\, e_j ,$$

where g_{dj} is replaced by $\beta_d\, e_j$, the slope of the linear regression of $[d_j]$ on e_j, etc. One can then compare the magnitudes of the interactions of the additive and dominance components with the range of environments by means of β_d and β_h, their linear regressions. In addition, the sensitivities to environmental variation of different inbred lines may be compared by means of their β_d s (Perkins and Jinks, 1968).

The accuracy of these regression equations is itself determined by the micro-environmental variation, that is, the sampling errors of g_{dj} and g_{hj}, which incorporate any genotype-environmental interaction within a single macro-environment. Such interaction in the open-field behaviour of rats was detected by Broadhurst and Jinks (1966), using analysis of variance of the variances. Subsequently Perkins and Jinks (1970) and Mather and Jinks (1971) specified the effects on the variances resulting from genotype interaction with the environmental variation within and between the families of each generation, in a manner similar to the way we defined the effects on the means of additive and dominance interaction with the macro-environments.

An extreme example of genotype-environmental interaction in the behaviour of *D. melanogaster* was reported by Ewing (1967); here the direction of genetical dominance was reversed between a circular runway where locomotor activity was measured over the first 2.5 minutes after the fly had been introduced to the apparatus, and a system of one-way vials where the distance travelled in 30 minutes was measured. By this time the arousing effects of transfer to the apparatus would have diminished, so that he was scoring spontaneous activity rather than reactivity (activity in response to stimulation), two traits known to differ in their genetical control (Connolly, 1967). Similarly in another genetical analysis of locomotor activity (Hay, 1972a), flies left in the original culture bottles instead of being reared in clean vials with fresh food medium for the seven days between eclosion and testing, showed genotype-dependent changes in activity over the three subsequent days of testing, even to the extent of a complete reversal both of the genetical dominance component and of maternal effects on performance. These results can be attributed to selective mortality and to differences in the effect of the poor conditions on spontaneous activity and reactivity. However the possibility remains that these interesting changes in performance were merely due to the active flies exhausting the available food more rapidly and fouling the culture bottles by depositing more "fly specks", themselves an index of reactivity to stimulation (Kaplan and Trout, 1969).

The present experiments were therefore designed on the hypothesis that the change in performance over the days of testing in the poor environment represented an acceleration of the normal changes in activity with age. Flies were maintained with adequate food at a controlled low density in the manner described below and samples tested every second day from 1—15 days after eclosion, in two types of apparatus differing principally in the intensity of the physical stimulation used to arouse the flies before the start of scoring. The eight successive tests in each apparatus may then be regarded as sampling eight different environments and by testing in each environment two long-inbred lines, 6 *C/L* and *Edinburgh*, as well as the reciprocal F_1 s between them, β_d and β_h can be estimated to compare the changes with age.

The other behaviour being considered in the genetical analysis is preening, the rubbing of the wings, legs and body surfaces, and it has not shown such extensive interaction between genotype and environment in *Drosophila*, possibly because of its function. Preening serves in all environments to fend off other flies and space them over the available food or egg-laying space (Connolly, 1968 and Hay, 1972b) and shows little genetical variation, apart from indirect effects attributable to differences in the density of flies in the cultures (Hay, 1972a). Thus, by starting with cultures of a constant size, any changes of preening with age may provide a crude indication of the number surviving at that time, without the disturbance caused to flies when removing them to be counted in the normal manner.

Method

The Rearing Conditions

For each type of apparatus a total of 3,840 flies from 6 *C/L*, *Edinburgh* and their F_1s were scored in two independent replicates. Only females were tested, since males have been found to be generally less robust and it was feared that insufficient might survive for the required fifteen days. All cultures were maintained by mass-mating in half pint bottles at 25 °C and tested at this temperature between 12.00 and 16.00 hours, when diurnal fluctuations in behaviour are minimal (Hay, 1972c). To avoid the problem of possible behavioural differences between flies emerging early or late from the pupal stage, all the flies for any one series of eight test days (one complete replicate) were collected at the end of a single 24-hour period of emergence, 30 females being taken from each of 32 cultures per genotype (four for each day of testing) and reared in a 3″ × 1″ vial. On each day testing was carried out in four 60-minute blocks to reduce the variation between flies in the time spent in the test apparatus before being scored. Although only 15 flies from the same culture were tested for each genotype in every block, it was found necessary to collect 30 flies at eclosion and transfer them to fresh medium on every second day, to ensure that sufficient survived to the fifteenth day.

Procedure

The flies were tested individually in one of two forms of container:

1. a 10 cm. length of glass tubing of 3.5 mm. internal diameter, the ends blocked by loose-fitting wooden plugs, or

2. an 18 mm. diameter polythene dish, 3 mm. deep, with a microscope cover-slip as lid. This lid, used originally to permit photography of the flies (Hay, 1972b), was not secured in any way and could easily be dislodged which meant that the flies in these containers could not be stimulated so vigorously prior to testing as those in the tubes.

Once the 60 flies had been introduced into their containers under light CO_2 sedation, they were assigned randomly to ten groups of six and a further 30 minutes allowed for recovery. Then, for each group in turn, the frequencies of preening and locomotor activity were noted over ten observations in the course of a one-minute period after stimulation by a sharp tap with the fingers against the sides of the small tray on which the groups of six containers rested. This stimulation aroused most flies from the inactivity which was common by this stage, but the containers prevented them from flying and non-directional illumination of 50 lux eliminated any possible effects of phototaxis. Further details of this procedure and its reliability are considered in Hay (1972d).

Results

As the data for each individual consisted of the proportions of activity and preening in the ten observations, each score was transformed to angles before the analysis to reduce the dependence of the variance on the mean. This transformation, however, is not primarily intended to remove genotype interaction within the macroenvironments, which is the aim of the type of rescaling used to eliminate any heterogeneity of variance among the non-segregating generations.

Table 1 summarizes the main findings apart from those concerned with the effects of age on performance. The mean transformed proportions and S.E.s of activity and preening, given in this table, are based on the 960 flies of each genotype from the eight days of testing and the two replicates. The variability of scores in the two types of apparatus were calculated within the samples of 15 flies scored for each genotype in every block of testing, with S.D.s derived as $\sqrt{2\ \text{var}^2/(\text{d.f.} - 1)}$ where d.f. = 3584. These variances may be compared with 78.14 (d.f. = ∞), the estimate of the theoretical variance of angular-transformed data given by the formula $820.7/(n + 0.5)$, where $n = 10$ in this case (Mosteller and Youtz, 1961). The additive and dominance genetical components, $[d]$, and $[h]$, were estimated for each replicate separately, to see whether the genetical components differed in their stability over replicates. For comparison between sets of data, $[d]$ was always calculated as $1/2\ (\overline{Edinburgh} - \overline{6\,C/L})$, retaining the sign, although $[d]$ must be positive by definition. $[h]$ was given as $\overline{F_1} - 1/2\ (\overline{Edinburgh} + \overline{6\,C/L})$, and the S.E.s for $[d]$ and $[h]$ were calculated from the parental and F_1 variances, as described by Mather and Jinks (1971).

Activity

Table 2a shows that the greatest influence on activity is the day of testing, which accounts for 41% of the variation. While the decline of activity with age is consistent over the two forms of apparatus (Fig. 1a and b), genotypic differences are very dependent upon the test conditions. All flies are less active in dishes, where the prior stimulation is reduced, but from Table 1 and the partitioning of the genotype sum of squares in Table 2b, clearly the inbred strain, 6 *C/L*, changes most between conditions.

Because of the magnitude of these interactions, each condition will be considered separately. Among the flies tested in tubes, neither β_d not β_h were signi-

Table 1. *Activity and preening data, averaged over all ages (after an angular transformation) and based on the proportion of each behaviour in ten observations*

	Activity		Preening	
	Tubes	Dishes	Tubes	Dishes
Means and S.E.s				
Edinburgh (E)	41.08 ± 0.56	39.42 ± 0.72	34.16 ± 0.45	31.39 ± 0.58
6 *C/L* (C)	54.29 ± 0.57	37.59 ± 0.84	22.02 ± 0.36	16.62 ± 0.37
E ♀ × C ♂	54.11 ± 0.52	48.28 ± 0.71	24.65 ± 0.37	25.76 ± 0.49
C ♀ × C ♂	54.29 ± 0.55	49.26 ± 0.66	24.13 ± 0.37	25.60 ± 0.46
Within-group variance (d.f. = 3584)	225.26 ± 5.32	408.21 ± 9.64	141.35 ± 3.34	212.51 ± 5.02
Estimates of $[d]$ and $[h]$ and the significance of their difference from zero				
Replicate 1 $[d]$	−5.64 ± 0.54***	+1.34 ± 0.77 n.s.	+5.80 ± 0.40***	+8.28 ± 0.49***
$[h]$	+5.94 ± 0.74***	+10.05 ± 1.03***	−3.12 ± 0.54***	+2.09 ± 0.68**
Replicate 2 $[d]$	−7.07 ± 0.59***	+0.48 ± 0.78 n.s.	+6.33 ± 0.42***	+6.49 ± 0.48***
$[h]$	+6.59 ± 0.82***	+10.47 ± 1.03***	−4.28 ± 0.56***	+1.26 ± 0.68 n.s.

n.s. = not significant; ** = $P < 0.01$; *** = $P < 0.001$.

Table 2 a. *Analysis of variance of activity with the percentage contributions of each item*

Item	d.f.	Mean square	Replicate interaction M.S.	Percentage variation
Condition (C)	1	105,987.2 n.s.	23,764.9***	14.5
Day (D)	7	62,622.7***	3,907.9***	41.3
Genotype (G)	3	53,308.8***	—	18.6
C×D	7	4,033.8 n.s.	1,786.1***	3.2
C×G	3	19,518.5**	—	13.5
D×G	21	1,623.3***	—	3.7
C×D×G	21	1,237.6***	—	5.2
Error variance	7168	316.7		

Table 2 b. *Partition of the genotype sum of squares*

Item	d.f.	Mean square	Condition interaction M.S.
Between F_1s	1	326.7 n.s.	154.2 n.s.
Between parents	1	28,463.1***	50,747.4***
Potence ($\overline{F_1}$-midparent)	1	131.136.2***	7,653.9***

n.s. = not significant; *** = $P < 0.001$.

Note. The within-group variance has been used as the error, except where the replicate interactions were significant.

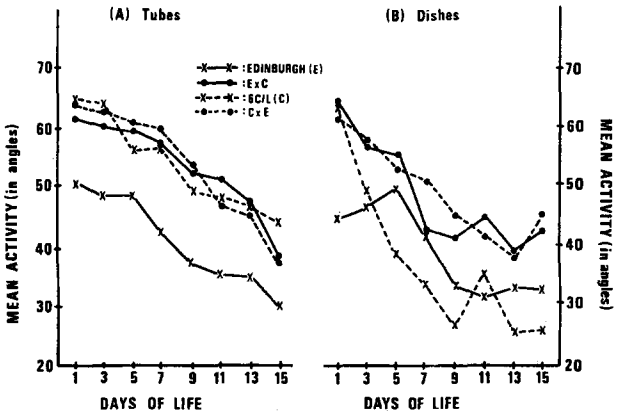

Fig. 1. Effects of age on the activity of female *Drosophila*, tested a) in tubes and b) in dishes. Each point is based on 120 flies and is the angular-transformed proportion of time spent in activity during the 60 seconds of observation. In the F_1s, the first-named parent is the mother

Table 3. *Activity in tubes — means and S.E.s on days 1 and 15 (each statistic based on 120 flies) expressed as the proportion (in angles) of all responses*

Genotype	Day 1	Day 15
Edinburgh (E)	51.88 ± 1.41	29.99 ± 1.48
6 *C/L* (C)	64.82 ± 0.96	29.65 ± 3.70
E♀×C♂	61.54 ± 1.15	44.18 ± 1.59
C♀×E♂	63.84 ± 1.08	38.83 ± 1.55

ficant, that is, neither the additive nor dominance components showed a linear change with age despite the large day × ×genotype mean square ($P < < 0.001$). Regressing the difference between the reciprocal F_1s ($\overline{E \times C} - \overline{C \times E}$) on e_j indicated an interaction of maternal effects with age, the joint regression over replicates having a slope of -0.1762 ± 0.0457. This is significant (d.f. = 12, $P = 0.001$) despite, it should be noted, a difference in 6 *C/L* as between replicates. By an appropriate partitioning of the replicates within days mean square (d.f. = 8, $P < 0.001$), all the significant replicate differences were traced to the erratic performance of the 6 *C/L* strain on day 5.

The nature of the maternal effect can be appreciated from Table 3; early in life, the level of activity in the F_1s is closer to that of their mothers, but subsequently this relationship is reversed. These flies were reared at a constant low density in uniform conditions from the day of emergence, so that these reciprocal differences must be due to factors operating earlier at the larval stage, where, for instance, competition for food occurs (Gale, 1964). Since Barnes (1968) has demonstrated maternal control in *D. melanogaster* over the percentage of eggs hatching, effects of the number of larvae utilizing the food would explain both the initial reciprocal difference in activity and its gradual disappearance at the adult stage when adequate food was available. However, an explanation in terms of larval competition would not predict the observed change to an apparent "paternal effect" on activity, and such possible influences as the more rapid exhaustion of the available food or fouling of the culture bottles by the active flies do not apply with this experimental design.

The variances display a similar pattern of change over the 15 days, the S.E.s in Table 3 showing the interaction between age and genotype. On day 1, the variances were heterogeneous ($\chi^2_3 = 18.24$, $P < 0.001$) because *Edinburgh*, the least active genotype, had a very large variance, but on day 15 the largest variances were found in those flies which were initially most active, namely the F_1s and in particular the inbred line, 6 *C/L*.

Turning to activity in dishes, a significant day ×genotype interaction was not due to reciprocal differences but to a change with age in the additive component ($\beta_d = -0.3325 \pm 0.1086$, d.f. = 12, $P < 0.01$). The strain 6 *C/L* was responsible for this increased difference between the inbred lines as the general level of activity dropped. The flies of this strain were equally active on the first day of testing in both forms of apparatus, but subsequently their activity as measured in dishes declined very rapidly

(Fig. 1 b). This change was accompanied by a disproportionate rise in variance from 266.08 ± 34.29 on day 1 to 455.60 ± 59.06 on day 15, the corresponding average variances in the other three genotypes being 366.20 ± 27.33 and 355.29 ± 26.52.

The linear additive genetical interaction with age would explain also the apparent overdominance for high activity in dishes, as indicated by the large values of $[h]$ relative to $[d]$ in Table 1. Bucio Alanis, Perkins and Jinks (1969) demonstrated that the difference between the means of the F_1 and larger parent could be defined as $([h] + g_{hj}) - ([d] + g_{dj})$. Since, in the present case, β_d is negative and dominance interaction (g_{hj}) with age is absent, then heterosis for high activity — where the F_1 mean exceeds that of either parent — is to be expected in data averaged over the eight days of testing.

The condition × day × genotype interaction in Table 2 can now be appreciated. Although dominance for high activity was common to both conditions of testing, comparing Fig. 1 a and b shows that the linear interaction of the reciprocal effect in tubes is only a minor influence on performance, relative to the β_d in dishes.

Preening

Unlike activity, preening does not alter with age and Table 1 shows that the type of apparatus influences only the performance of the inbred lines. Because the F_1s are unaffected, the direction of dominance ($[h]$) is reversed between the conditions. It will be recalled that Ewing (1967) also found the direction of dominance depended upon the type of apparatus, presumably for the same reason, namely that if the F_1s are more responsive to the stimulation their performance will be less dependent upon differences in the intensity of the initial stimulus applied to the flies.

In tubes, the only genotype interaction in the analysis of the means involved the replicates. The 6 *C/L* strain was again responsible, although the interaction here took a very different form from that found in the analysis of activity in tubes. The regressions of the 6 *C/L* means for each day on the values 1–8 were −0.8432 ± 0.1793 and +1.2455 ± 0.3120 for the two replicates respectively, and differ significantly from each other ($P < 0.001$).

Despite the absence of genotype-dependent changes in the means with age, such effects were found among the variances. The variance of *Edinburgh* increased significantly ($P < 0.01$ on a variance ratio test for 112 and 112 d.f.) from 148.09 ± 19.20 on day 1 to 260.51 ± 33.76 on day 15, while age did not affect the variances of the other three genotypes.

Preening in dishes showed genotype interaction of the means with replicates in an analysis which illustrates the relative stability of the additive and dominance components. The linear regressions of the daily means of 6 *C/L* and of both F_1s on 1–8 differed significantly between replicates (all $P<0.01$), but a partition of the genotype sum of squares (Table 4) shows the potence level (the difference between $\overline{F_1}$ and midparent) does not alter. This is also indicated by the estimates of $[d]$ and $[h]$ and their S.E.s in Table 1; $[h]$ is constant in spite of a significant change ($P < 0.01$) in $[d]$ between replicates.

Table 4. *Preening in dishes — partition of the genotype sum of squares*

Item	d.f.	Mean square	Replicate M.S.
Between F_1s	1	13.0 n.s.	227.7 n.s.
Between parents	1	104,713.8***	1537.9**
Potence	1	2,686.2***	166.1 n.s.

n.s. = not significant; ** = $P < 0.01$; *** = $P < 0.001$.
The basic error term is the within-group M.S. of 212.5 for 3584 d.f.

The greater stability over environments of preening in the hybrids also applies to the microenvironmental effects. Thus, unlike the performance in tubes where only *Edinburgh* increased in variability over the fifteen days, the variances of both inbred lines changed on average by +117.61 ± 43.48, while the F_1 variances were unaltered (−4.67 ± 38.12).

Discussion

The analyses demonstrate that the genotypes differ in their sensitivity to many kinds of environmental change. That even the replicate variation is largely strain dependent has particular implications for the tests of significance based on replicate interactions which are often used in "mixed" model analyses of variance (Snedecor and Cochran, 1967). The F_1s proved more stable than the inbred lines in all respects, their insensitivity to age and apparatus effects leading, for instance, to the overall heterosis for high activity.

Instead of rescaling the data to eliminate the heterogeneity between genotypes in the variances within each environment, these variances can be considered in the light of data on the viability of *Edinburgh* and 6 *C/L*, given in Hay (1972a), and the possible negative correlation of viability with variability mentioned in the introduction. The high initial variability of *Edinburgh* on both activity and preening may reflect its poor larval-adult survival rate of 17.0% ± 2.1%, compared with 81.2% ± 2.5% in 6 *C/L*. In addition, in good rearing conditions, the adult mortality of *Edinburgh* was higher — the percentage of females surviving for 7 days after eclosion was 49.3% ± 4.2% in *Edinburgh* and 79.3% ± 5.6% in 6 *C/L* — and Dobzhansky and Levene (1955) found that genotypes with a poor viability also showed more variability in the number of flies alive in each culture.

Therefore, in view of the relationship between culture density and preening (Connolly, 1967; Hay, 1972a), the increase with age in the variability of preening among the flies tested in tubes, which was confined to *Edinburgh*, can be attributed to a greater variability between the cultures of this strain in the number of adults surviving.

But the variability among the 6 *C/L* flies for preening in dishes increases to the same extent as that of *Edinburgh* and 6 *C/L* also has the most marked rise in variability for activity under both testing conditions. Two factors may contribute to these results. First, the greater variability of 6 *C/L* may relate to poorer viability later in life. The "rate of living" theory of ageing (Shaw and Bercaw, 1962) proposes that the higher the metabolic rate, the faster the ageing process, so that those flies most active early in life should be less vigorous and less viable at a later stage. Such differences in adult viability may become evident only in poor conditions, (Marinkovic and Wattiaux, 1967), where indeed the mortality among 6 *C/L* flies is higher than among *Edinburgh* (Hay, 1972a). Assuming that high activity here is indicative of a high metabolic rate, this theory of ageing would predict the observed reversal over the 15 days of the maternal effect on the activity of the F_1s and also, assuming that viability and variability are connected, the considerable rise in the variability of activity in 6 *C/L*. Furthermore, despite the heterosis for high activity, the rise in the F_1s' variance should be smaller, because hybrids, as well as being less variable in performance, often have a viability far superior to that of their inbred parents (Dobzhansky and Levene, 1955; Parsons, 1966).

A second factor, reactivity, is necessary to explain the increased variability among the 6 *C/L* flies when tested in dishes. Before either activity or preening can occur, the flies must be aroused from the inactive state common by the end of the 30-minute recovery period. The initial stimulus which serves this purpose is less in the dishes and the 6 *C/L* strain is most influenced; whereas inactivity, expressed as an angular-transformed proportion of all responses in the eight testing sessions, increased in the other three genotypes from 24.09 $\pm$ 0.93 in tubes to 28.69 $\pm$ 0.69 when they were tested in dishes, the change in 6 *C/L* was from 25.37 $\pm$ 0.60 to 46.39 $\pm$ 0.93. Presumably greater inter-individual variability (the within group variances in Table 1) will also result from the decreased stimulation, since the probability of a fly being excited into movement must depend more on chance factors than on the external stimulus. Similarly, the changes with age, where activity but not preening decreased and the individual variability on both measures increased, are compatible with a declining responsiveness. If it is spontaneous activity rather than reactivity that is being measured in the dishes, then performance here should be closely related to that in the usual culture bottles where there is little extraneous stimulation and where activity and preening are essential to avoid becoming mired by the accumulating debris and secretions (Hay, 1972a). As mentioned already, the adult 6 *C/L* had a poor viability in these conditions but the high mortality among all inactive flies made it difficult to relate strain differences in behaviour to survival. Some evidence comes from the present experiment where fresh culture vials were used; here the 6 *C/L* flies tested in dishes show a very low level of activity and preening especially after the 9th day (Fig. 1b), the time when, in the earlier work, its mortality in crowded conditions began to increase above that of *Edinburgh*.

It is not yet possible to conclude whether variability only reflects viability differences, or whether sensitivity to the environment itself affects viability. Marinkovic and Wattiaux (1967) have evidence for a selective advantage in the ability of *D. pseudoobscura* to withstand environmental fluctuations, leading to the increased fitness of heterozygotes. This issue is of particular relevance to many psychogenetic studies based on artificial selection since, when complete homozygosity is approached, viability decreases (Siegel, 1967) and the variability of performance between generations may increase, as Connolly (1966) reported in his activity selection lines. In contrast, Dobzhansky and Montagu (1947) argued for the existence in behaviour of an "adaptive trait of environmental responsiveness". A good illustration of this distinction is provided by Band (1964) who, in studying the viability of *D. melanogaster*, found that heterozygotes had a superior capacity to adapt to altered temperatures, but displayed less "error" variation within any one temperature.

It is therefore dangerous to speak as generally as Caspari (1958) has done, of the possibility that behavioural heterosis is expressed by increased variability. Although it has long been recognised in the biometrical genetics of non-behavioural characters that such variability and genotype-environmental interaction in general can distort estimates of heritability (Mather and Jinks, 1971), this point is only now being appreciated in psychogenetics. Hirsch (1970) refers to this variability as "unreliability", while Newell (1970) calls genotype-environmental interaction "the most serious problem in behavior genetics, because its effect on estimates of genetic parameters is unknown". Here, biometrical genetical techniques have been introduced which allow the analysis of such genotype-environmental interaction but highlight one drawback common to so many behavioural measures. That is, the interpretation of the results may be ambiguous because of uncertainty regarding what is being measured. In this case, the measure of activity initially depends

largely upon the responsiveness to stimulation, but in older flies and in an apparatus where stimulation is reduced, it is more closely related to the level of spontaneous activity. This sensitivity of behaviour to environmental and procedural factors makes it all the more essential to adopt a systematic approach to variability, rather than striving to eliminate it from the analysis.

Acknowledgements

This work was carried out during the tenure of a British Science Research Council postgraduate studentship. The author thanks Dr. M. J. Kearsey for his advice and encouragement throughout the study, and Professors P. L. Broadhurst and J. L. Jinks for their valuable comments on the manuscript.

Literature

1. Band, H. T.: Genetic structure of populations, III. Natural selection and concealed genetic variability in a natural population of *D. melanogaster*. Evolution **18**, 384–404 (1964). — 2. Barnes, B. W.: Maternal control of heterosis for yield in *D. melanogaster*. Heredity **23**, 563–572 (1968). — 3. Broadhurst, P. L., Jinks, J. L.: Biometrical genetics and behavior: reanalysis of published data. Psychol. Bull. **58**, 337–362 (1961). — 4. Broadhurst, P. L., Jinks, J. L.: Stability and change in the inheritance of behaviour in rats: a further analysis of statistics from a diallel cross. Proc. Roy. Soc. **B**, **165**, 450–472 (1966). — 5. Bucio Alanis, L., Hill, J.: Environmental and genotype-environmental components of variability, II. Heterozygotes. Heredity **21**, 399–405 (1966). — 6. Bucio Alanis, L., Perkins, J. M., Jinks, J. L.: Environmental and genotype-environmental components of variability, V. Segregating generations. Heredity **24**, 115–127 (1969). — 7. Caspari, E.: The genetic basis of behavior. In: Behavior and Evolution, ed. by A. Roe and G. G. Simpson, 103–127. New Haven: Yale U.P. 1958. — 8. Connolly, K. J.: Locomotor activity in *Drosophila*, II. Selection for active and inactive strains. Anim. Behav. **14**, 444–449 (1966). — 9. Connolly, K. J.: Locomotor activity in *Drosophila*, III. A distinction between activity and reactivity. Anim. Behav. **15**, 149–152 (1967). — 10. Connolly, K. J.: The social facilitation of preening behaviour in *D. melanogaster*. Anim. Behav. **16**, 385–391 (1968). — 11. Dobzhansky, T. H., Levene, H.: Genetics of natural populations, XXIV. Developmental homeostasis in natural populations of *D. pseudoobscura*. Genetics **40**, 797–808 (1955). — 12. Dobzhansky, T. H., Montagu, M. F. A.: Natural selection and the mental capacities of mankind. Science **105**, 587–590 (1947). — 13. Ewing, A. W.: Genetics and activity in *D. melanogaster*. Experientia **23**, 330–335 (1967). — 14. Gale, J. S.: Competition between three lines of *D. melanogaster*. Heredity **19**, 681–699 (1964). — 15. Hay, D. A.: Genetical and maternal determinants of the activity and preening behaviour of *D. melanogaster*. Heredity **28**, 311–336 (1972a). — 16. Hay, D. A.: Recognition by *D. melanogaster* of individuals from other strains or cultures: support for the role of olfactory cues in selective mating? Evolution, **26**, 171–176 (1972b). — 17. Hay, D. A.: Behavioural rhythms in cultures of immature *D. melanogaster*. Experientia, **28**, 922–923 (1972c). — 18. Hay, D. A.: Effect of genetic variation and culture conditions on the social behaviour of *D. melanogaster*. Behavior Genetics, in press (1972d). — 19. Hirsch, J.: Behavior-genetic analysis and its biosocial consquences. Seminars in Psychiatry **2**, 89–105 (1970). — 20. Jinks, L. J., Jones, R. M.: Estimation of the components of heterosis. Genetics **43**, 223 to 234 (1958). — 21. Kaplan, W. D., Trout, W. E.: The behavior of four neurological mutants of *Drosophila*. Genetics **61**, 399–409 (1969). — 22. Marinkovic, D., Wattiaux, J. M.: Genetic loads affecting longevity in natural populations of *D. pseudoobscura*. Nature **216**, 170–171 (1967). — 23. Mather, K., Jinks, J. L.: Biometrical Genetics (2nd ed.). London: Chapman and Hall 1971. — 24. Mosteller, F., Youtz, C.: Tables of the Freeman-Tukey transformations for the binomial and Poisson distributions. Biometrika **48**, 433–440 (1961). — 25. Newell, T. G.: Three biometrical genetic analyses of activity in the mouse. J. comp. physiol. Psychol. **70**, 37–47 (1970). — 26. Parsons, P. A.: The genotypic control of longevity in *D. melanogaster* under two environmental regimes. Aust. J. Biol. Sciences **19**, 587–591 (1966). — 27. Parsons, P. A., Kaul, D.: Mating speed and duration of copulation in *D. pseudoobscura*. Heredity **21**, 219–225 (1966). — 28. Perkins, J. M., Jinks, J. L.: Environmental and genotype-environmental components of variability, III. Multiple lines and crosses. Heredity **23**, 339–356 (1968). — 29. Perkins, J. M., Jinks, J. L.: Detection and estimation of genotype-environmental, linkage and epistatic components of variation for a metrical trait. Heredity **25**, 157–177 (1970). — 30. Roberts, R. C.: Implications of behavior genetics for genetics. In: Behavior-genetic Analysis, ed. J. Hirsch, 340–343. N.Y.: McGraw Hill 1967. — 31. Shaw, R. F., Bercaw, B. L.: Temperature and life-span in poikilothermous animals. Nature **196**, 454–457 (1962). — 32. Siegel, I. W.: Heritability and threshold determinations of the optomotor response in *D. melanogaster*. Anim. Behav. **15**, 299–306 (1967). — 33. Snedecor, G. W., Cochran, W. G.: Statistical Methods (6th ed.). Ames: Iowa State Univ. Press 1967.

Received September 20, 1972
Communicated by H. Stubbe

Dr. David A. Hay
Lecturer in Genetics
Department of Genetics & Human Variation
La Trobe University
Bundoora, Victoria (Australia 3083)