Theor Appl Genet (1989) 78: 243–248

Environmental sensitivity and heterosis for egg laying in *Drosophila melanogaster*

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Received May 31, 1988; Accepted April 12, 1989
Communicated by J.S.F. Barker

Summary. Genotype × temperature interactions for egg laying were studied in *Drosophila melanogaster* using two sets of half diallel crosses: one between inbred lines of the same geographic origin, and the other between established laboratory, newly derived inbred lines from different geographic origins. The sensitivity of most genotypes to changes in temperature was adequately described as a linear regression of mean in temperature. The regression coefficients (linear sensitivities) were heterogeneous between genotypes. Hybrids were more affected by temperature variation than were inbreds. All the heterogeneity of linear sensitivities was accounted for by a linear function of the genotypic means, which strongly suggests that a scale effect is responsible for the differences in sensitivity to temperature. In contrast, no general relationship was found between standard error deviation (sensitivity to small environmental changes) and mean performance between genotypes, although hybrids tended to be less variable than inbreds. This shows that the sensitivity to environmental variation depends not only on the genotype, but also on the nature of the environmental variation. The variability within temperatures may be affected by the general homeostasis of individual genotypes, while the variability between temperatures could be the result of genes directly affecting the trait and their multiplicative interaction with the environment.

Key words: Genotype × temperature interaction – Heterosis – Egg laying – *Drosophila melanogaster*

Introduction

The magnitude of heterosis is conditioned by the environment (for a review, see Barlow 1981). According to the concept of genetic homeostasis (Lerner 1954), heterozygotes are likely to be better buffered against environmental variation than homozygotes. Consequently, heterozygotes might be expected to show greater heterosis in non-optimal, rather than optimal environments.

Hybrids are less variable between environments than inbreds for most traits and organisms; greater heterosis was found under extreme environmental conditions than under optimal ones (Barlow 1981; Tachida and Mukai 1985; Clare and Luckinbill 1985).

It was also found that hybrids are less variable (within a given environment) than their parental strains in *Drosophila* for a variety of traits: survival, size and developmental time (Robertson and Reeve 1952), fecundity (Robertson and Reeve 1955; Domínguez and Albornoz 1987), wing and thorax length and percentage emergence (Tantawy 1957).

Unlike this general trend, Connolly and Jinks (1975) found that the level of heterosis for growth rate in *Schizophyllum commune* increased with improved environments in a study of genotype-temperature interaction. Sang (1964) found that under quasi-optimal nutritional conditions, *D. melanogaster* hybrids were generally superior to inbreds for developmental rate, but they showed no special advantages under no-optimal conditions. This seems to be generally the case for growth and nutrition, while the opposite appears to hold true for other traits and other environmental variables in a variety of organisms (Barlow 1981).

In a previous study, Domínguez and Albornoz (1987) found that *D. melanogaster* hybrids displayed less variation within a given environment than their parents in relation to their egg-laying rate. This agrees with the greater homeostasis of hybrids but, in contrast, they were more affected by major environmental changes (low temperature and high larval density), which resulted in

higher heterosis as the environment approached optimal conditions. These results are comparable to those of Sang (1964), who found similar heterosis for *D. melanogaster* developmental rate under normal or modified diets. Nevertheless, Ruban et al. (1988) reported a higher percentage of heterosis for egg laying on a poor nutritional diet than on a normal one. However, when heterosis was expressed in those units in which the trait was measured, there was no difference.

The problem of variable expression of heterosis in different environments must be seen from a general point of view within the topic of genotype-environment interaction, since the differences in sensitivity between hybrids and inbreds might not be related to heterozygosity per se.

This paper describes a study of heterosis-temperature interaction for egg laying. The responses of individual genotypes over a range of four temperatures were examined, in order to interpret the differences in heterosis from the general view of the interconnection of genes and environment. In the same way, attention was paid to the relationships between genotypic mean and standard deviation, which would throw light on the differences in homeostasis between genotypes.

Materials and methods

The study was carried out on 11 inbred lines derived from Spanish wild populations: Teverga-3, Proaza-3 and 9 lines derived from a population caught in Sandiche (S-3, S-4, S-5, S-8, S-11, S-12, S-13, S-14 and S-16); and six inbred wild-type laboratory stocks provided by the Umeå Drosophila Stock Center: Canton S, Oregon R, Hampton Hill, Crkwenica, Israel and Kreta 75. Inbred strains from the same and different geographical origins were used, since the stability shown by a hybrid depends upon the coadaptation of the parental genotypes (Mather 1955).

Two 9×9 half diallel sets of matings were performed: one with the lines from the Sandiche population (set 1), and another with the remainder and S-3 (set 2). In each set, the mating scheme was designed so that each line was used with equal frequency, both as male and female parents. Forty males and 40 females were mated to produce each of the 45 genotypes in each set. Their offspring were reared at 24 °C. Three pairs, formed by virgin females from each genotype and males from a pooled sample, were placed in different vials at 17°, 20°, 24° and 28 °C on their first day of life. Every day, from the 2nd to the 7th, flies were transferred to fresh medium and the eggs laid were counted. Five egg-laying vials were tested per inbred, and two per hybrid genotype, at each temperature to obtain the same number of degrees of freedom for variances of both inbreds and hybrids. The basic value obtained from each vial was the daily mean egg production per female, which is the result of dividing the total number of eggs laid in a given vial during the period considered by the number of females (3) and the number of days of laying (6). For more details, see Domínguez and Rubio (1986).

The relation between means and standard deviations within genotypes and temperatures (which are a measure of sensitivity of genotypes to small environmental changes) were tested by an analysis of regression. Standard error deviations, averaged over the four temperatures, were regressed on genotypic means, separately for inbreds and crosses (since crosses are usually less variable than inbreds), from the same (set 1) or different origin (set 2).

The genotype-temperature interaction was analysed following Perkins and Jinks (1968a). This focuses attention on the effect of the environmental differences by the way in which each individual genotype manifests itself. A regression approach to the analysis of genotype-environmental interactions was carried out; the mean performance (y_{ij}) of the i^{th} genotype in the j^{th} environment is described by the model:

$$y_{ij} = \mu + d_i + \beta_i z_j + \delta_{ij}$$

where

μ = grand mean over all genotypes and environments;
d_i = genetical contribution of the i^{th} genotype;
β_i = regression coefficient of the i^{th} genotype for the regression of y_{ij} on z_j;
z_j = independent assessment of the effect of the j^{th} environment;
δ_{ij} = deviation from its regression on z_j of the i^{th} genotype in the j^{th} environment.

Following this approach, three aspects of phenotype are recognised: (1) mean expression ($\mu + d_i$), (2) linear sensitivity to environmental change (β_i) and (3) non-linear sensitivity to change in environment $MS_i = \Sigma_{j-1,S}\, \delta^2{}_{ij}/(S-2)$.

The existence of genotype-environment interaction is revealed by the heterogeneity of linear sensitivities (β_i) and the deviations of the regression (δ_{ij}).

The mean of all genotypes in each environment ($\bar{y}_j$) was used to estimate the environmental component (z_j). It is clear then that the environmental values used on the X axis of the regression analysis are not independent of the phenotypic variable regressed on them, as already pointed out (Freeman and Perkins 1971). Nevertheless, it has been shown that the conclusions drawn from the data using both dependent and independent variables have not usually been dependent on the method used to quantify the environments (Fripp 1972; Mather and Caligari 1974).

The *log* transformation was discarded because it increased the heterogeneity of error variance (MS with elemental cell); besides, the standard deviation of the transformed variable is more affected by changes in mean level ($r = -0.51$, $p < 0.001$ for set 1 and $r = -0.59$, $p < 0.001$ for set 2) than that of the initial variable itself ($r = 0.21$, $p < 0.01$ for set 1 and $r = 0.23$, $p < 0.01$ for set 2). Therefore, we can suppose that arithmetic averages over the *log* scale are not good estimates of the means for any particular group of measurements, since the actual effects of the factors under study can thus be masked. Taking the above considerations into account, we have found the original scale to be the most relevant and the most understandable.

Results

Means and variances (either between or within genotypes) of hybrids and inbreds rose with temperature, at least up to 24 °C (Table 1), showing a relation between the two parameters. Despite the equal or lower sensitivity of hybrids to environmental variation within temperature, the increase in egg laying over temperatures is greater for hybrids than for inbreds. This leads to a greater heterosis (in number of eggs) at higher tempera-

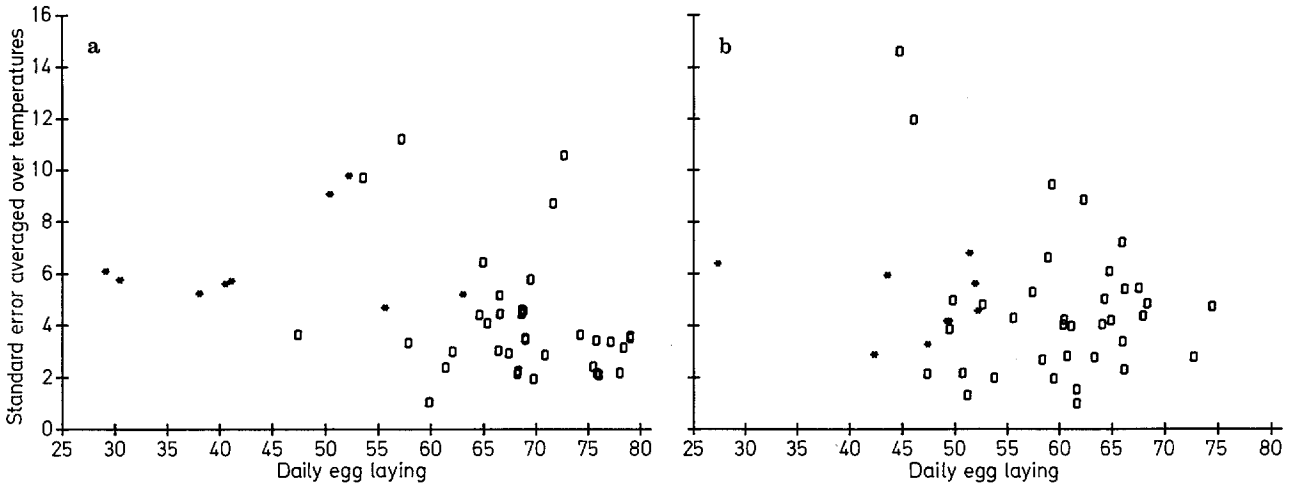

Fig. 1 a and b. Standard error averaged over temperatures for each of the 45 genotypes (hybrids: o, inbreds: *) from each set plotted against mean performances; **a** = set 1; **b** = set 2

Table 1. Means and components of variance of inbreds and hybrids within temperature. Degrees of freedom are shown between brackets

	Temperature			
	17 °C	20 °C	24 °C	28 °C
Set 1				
Inbreds				
Mean	12.75	41.16	59.56	66.93
Var. between genot. (8)	28.83	129.12	239.87	255.60
within genot. (36)	5.65	41.47	69.59	122.20
Hybrids				
Mean	19.47	62.19	86.56	105.84
Var. between genot. (35)	16.38	58.78	86.15	89.25
within genot. (36)	8.27	25.17	49.04	47.87
Heterosis ±SE	6.72±0.49	21.03±1.13	27.00±1.49	38.91±1.75
Set 2				
Inbreds				
Mean	12.39	40.17	58.11	73.81
Var. between genot. (8)	11.76	50.28	110.53	112.17
within genot. (36)	5.12	18.81	63.15	52.83
Hybrids				
Mean	12.69	54.15	76.36	96.89
Var. between genot. (35)	20.63	46.25	72.80	91.51
within genot. (36)	12.47	22.84	67.13	63.93
Heterosis ±SE	0.30±0.54	13.98±0.86	18.25±2.34	23.08±1.44

$$\text{Heterosis } \frac{F_1 - MP}{SE = \sqrt{(\text{var.}_{\text{within inbr}}/45) + (\text{var.}_{\text{within hybr}}/72)}}$$

tures than at lower ones. This general survey points to some interrelations between mean egg laying and sensitivity to either minor or major changes in the environment, which must be further analysed.

Standard deviations averaged for temperatures were plotted against genotypic means (Fig. 1). Regressions, calculated separately for hybrids and inbreds, were not significant. In set 1, hybrids were less variable than inbreds ($t' = 3.08$, $p < 0.01$), while no differences were observed in set 2. It is worth noting that error variance is not correlated with genotypic mean, while a positive relation between the two parameters was observed when temperature was the factor that varied (Table 1).

We can now examine the genotype-temperature interaction. The linear sensitivity (b_i) was calculated, for each genotype (i), as the regression of the means (y_{ij}) at the

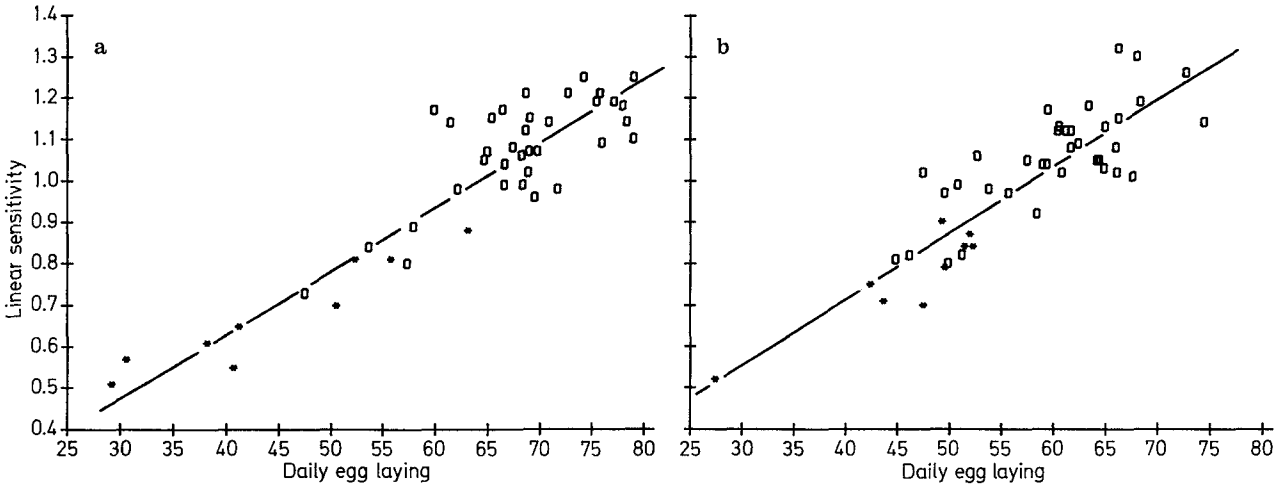

Fig. 2a and b. Linear sensitivities for each of the 45 genotypes (hybrids: o, inbreds: *) from each set plotted against mean performances; **a** = set 1; **b** = set 2

Table 2. Test of heterogeneity between genotypes of linear sensitivities to temperature

Source of variation	*df*	MS	*F*
Set 1			
Heterogeneity of regressions:			
Hybrids vs. inbreds	1	4,156.08	72.71[b]
Among hybrids	35	55.71	2.27[a]
Among inbreds	8	63.49	2.59[a]
Remainder	90	24.51	
Set 2			
Heterogeneity of regressions:			
Hybrids vs. inbreds	1	2,406.89	44.85[b]
Among hybrids	35	54.92	2.61[b]
Among inbreds	8	48.06	2.29[a]
Remainder	90	21.03	

[a] $p<0.01$; [b] $p<0.001$

Table 3. Variance analysis of regression of linear sensitivities on mean performance

Source of variation	*df*	*b*	MS ($\times 10^{-3}$)	*F*
Set 1				
Regression	1	0.0148	1,555.62	223.51[a]
Remainder	43		6.96	
Set 2				
Regression	1	0.0157	993.99	165.11[a]
Remainder	43		7.11	

[a] $p<0.001$

four temperatures on the corresponding averages obtained from all 45 genotypes ($\bar{y}_j$) from each set. A linear regression accounted for all the variation over temperatures for 36 genotypes in set 1, and 37 genotypes in set 2. The deviations from linear regression were significant for the remaining 9 genotypes (6 at the 0.05, 1 at the 0.01 and 2 at the 0.001 level) in set 1, and 8 genotypes (6 at the 0.05, 1 at the 0.01 and 1 at the 0.001 level) in set 2.

Linear sensitivities were heterogeneous in the two sets (Table 2), the heterogeneity being mainly accounted for by the difference between the coefficients of hybrids and inbreds.

In Fig. 2, the linear sensitivity of each genotype (b_i) is plotted against its mean performance ($\bar{y}_i$). These two parameters are obviously related. The analysis of regression (Table 3) shows that linear sensitivity is a function of mean performance.

Following Mather (1975), linear sensitivities of the different genotypes will be $\bar{y}_i/\bar{y}$ ($\bar{y}_i$ being the mean of line i over temperatures, and $\bar{y}$ the general mean), when the relation between genotype and environment is multiplicative. Thus, the regression slope of individual sensitivities (b_i) on the genotypic means ($\bar{y}_i$) will be $1/\bar{y}$. The expected slopes for set 1 and set 2 are 0.0157 and 0.0175, respectively, which did not differ from the actual observed slopes (0.0148 ± 0.0010 and 0.0157 ± 0.0014).

Given such a relation between sensitivities and genotypic means it follows that:

$$y_{ij} = \mu + d_i + (\mu + d_i)\, z_j/\mu + \delta_{ij} =$$
$$= (\mu + d_i)\,(\mu + z_j)/\mu + \delta_{ij}$$

The proportion of variance between means being explained by the multiplicative effect ($\bar{y}_i \bar{y}_j/\bar{y}$) is 98% for both set 1 and set 2.

Discussion

Before considering the variations of heterosis with temperature, the nature of genotype-by-temperature interac-

tion must be explored. The sensitivity of most genotypes to changes in temperature was adequately described by a linear relation with the overall effect of the environment (linear sensitivity). Therefore, the differences in linear sensitivity between genotypes accounted for most of the genotype-temperature interaction.

The results obtained from the two categories of crossing are similar. Linear sensitivity is positively related to the genotypic mean itself. That is, the greater the mean egg laying of a given genotype, the greater its sensitivity to changes in temperature. Thus, our results agree with those found in most random collections of genotypes withing several species for a number of traits. Examples are: yield in maize (Eberhart and Russell 1966), final height in *Nicotiana rustica* (Perkins and Jinks 1968a), flowering time and leaf number in *Arabidopsis thaliana* (Westerman and Lawrence 1970; Westerman 1971), growth rate in *Schizophyllum commune* (Fripp and Caten 1973; Connolly and Jinks 1975) and yield in Spring oats (Lawes 1977). There are also some exceptions where genetic variability for enviromental sensitivity was found to be independent of mean performance (Perkins and Jinks 1968b; Westerman and Lawrence 1970; Paroda and Hayes 1971; Powell and Phillips 1984).

Fripp and Caten (1973) interpreted the positive correlations between mean performance and linear sensitivity as being due to pleiotropy. Although it would be difficult to discard this interpretation, our study shows that a multiplicative effect of genotype and temperature can explain the positive relationship between linear sensitivity and mean performance, since differences in sensitivity are entirely accounted for by differences in mean performance. This might have a biological significance; traits related to production such as weight, growth rate, egg laying, etc. are best thought of as having a geometrical rather than arithmetical variation: the increment added by change of environment may be proportional to the genotype value. In the case of egg laying, differences in daily output might be a reflection of differences in nutrient conversion which would change with temperature as a function of metabolic rate.

The relation between mean performance and linear sensitivity might be regarded as a scale effect in the range of temperatures studied. Outside this range, the egg-laying rate declines (Ashburner and Thompson 1978) and the relation between the two variables might be different.

Hybrids were more sensitive than inbreds to temperature changes. These results contrast with most other studies on heterosis-environment interaction. However, similar results are rather general for growth and nutrition (Barlow 1981) and were also found in a previous study on egg laying and developmental density in Drosophila (Domínguez and Albornoz 1987).

In the present study, differences in sensitivity can readily be ascribed to differences in mean egg laying and are, thus, only apparent. They are due to the multiplicative effects of genotype and temperature. The kind of traits and environmental variables which in other studies have led to similar results (growth and nutrition) suggests that they might also be subjected to multiplicative interaction.

Turning now to the relationship between mean and standard error deviation, where both rise with temperature, this agrees with the relation between mean and linear sensitivity and could be interpreted, in the same way, as a scale effect. Nevertheless, no such relation between the two parameters was observed when attention was focused on differences between genotypes. This shows that the interaction between small uncontrolled environmental change and genotype is different from the temperature-genotype interaction, and agrees with Sang (1964), in that the sensitivity of genotypes to minor environmental variation cannot be simply extended to major changes.

Hybrids of lines from the same geographical origin showed lower sensitivity than inbreds, while no differences were found between hybrids and inbreds from different origins. This can be due to the greater coadaptation of the hybrids from lines of the same origin (Mather 1955; Dobzhansky and Levene 1955).

It is clear that both scale effects and differences in hybrid and inbred response to environmental changes would be involved in the heterosis-environment interaction. Therefore, it would be difficult to compare experiments with different organisms, traits and environmental variables if the nature of the genotype-environment interaction is unknown.

Acknowledgements. We thank Dr. E. Alcorta for providing the inbred lines from the Sandiche's population.

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