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Phenotypic plasticity for life history traits in *Drosophila melanogaster*. II. Epigenetic mechanisms and the scaling of variances

M. D. Gebhardt[1] and S. C. Stearns

Zoologisches Institut, Rheinsprung 9, CH-4051 Basel, Switzerland

Key words: Phenotypic plasticity; reaction norm; physiological mapping function; epigenetic mechanism; variance components.

Abstract

We manipulated developmental time and dry weight at eclosion in 15 genotypes of *Drosophila melanogaster* by growing the larvae in 9 environments defined by 3 yeast concentrations at 3 temperatures. We observed how the genetic and various environmental components of phenotypic variation scaled with the mean values of the traits.

Temperature, yeast, within-environmental factors and genotype influenced the genotypic and environmental standard deviations of the two traits in patterns that point to very different modes of physiological and developmental action of these factors. Since different factors affected the environmental and genetic components of the phenotypic variation either in parallel or inversely, we conclude that environmental heterogeneity may have small or large effects on evolutionary rates depending on which factors cause the heterogeneity. The analysis also suggests that the scaling of variances with the mean is not as trivial as is often assumed when coefficients of variation are computed to "standardize" variation.

Introduction

Modern evolutionary biology has been described as a synthesis between genetics and Darwinism (e.g. Huxley, 1942). However, decades after the synthesis had been

[1] Current address: Swiss Federal Office of Public Health, Epidemiology and Infectious Diseases, CH-3097, Bern/Liebefeld, Switzerland

initiated, most scientific progress was still achieved in separate fields that either focussed on genotypes, as in population genetics, or on phenotypes, as in life history theory. The study of the intermediate structure (Stearns, 1989), which determines the relationship between genotype and phenotype is still in its infancy (Lewontin, 1974) and therefore a pressing problem in evolution. Phenotypic plasticity provides an important approach to this problem because it suggests experimental ways to bridge the gap between genotype and phenotype, keeping both in view while avoiding the detail of molecular or physiological approaches.

Often a plastic trait can be changed by different environmental factors. In the accompanying paper, for example, we described how developmental time and weight at eclosion of *Drosophila melanogaster* change with shifts in the temperature and yeast concentration provided during larval development (Gebhardt and Stearns, 1993). Here we ask whether different factors that act during ontogeny and have a similar effect on the phenotype do so by affecting the same pathways in the epigenetic system. We take up a conceptual framework first developed by Rendel (1959) and later extended by Scharloo (e.g. 1987 for a review), who coined the term genotype-environmental factor/phenotype mapping function (GEPM). The concept is related to reaction norms, although it also includes the possibility that shifts in genetic factors can change the phenotype according to the same function, such as segregation or shifts in the genetic background by directional selection.

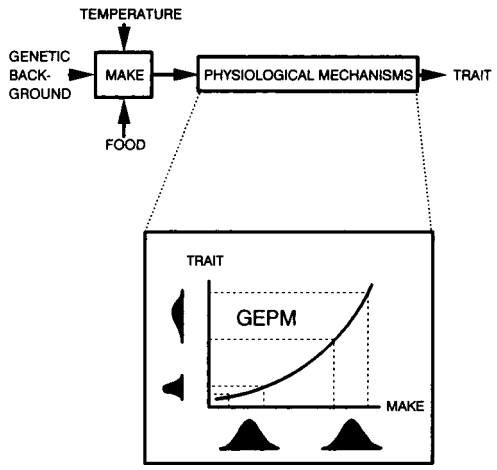

Fig. 1. The concept of Genotype Environmental factor/Phenotype Mapping functions (GEPMs) as a tool for describing physiological and developmental processes (Scharloo, 1987). A trait is thought to depend on an underlying hypothetical variable, "Make" (Rendel, 1967), which in turn depends on various environmental and genetic factors. The shape of a GEPM depends on the structure of the physiological mechanisms and causes a characteristic relationship between the mean and the standard deviation of the trait. The example shown is a convex increasing function which would produce a positive correlation between mean and standard deviation of the trait.

In this model, the expression of the trait is thought to depend on an underlying physiological variable (Fig. 1). Rendel (1959, 1967) was not specific about this variable and called it "Make", but it could be the concentration of a morphogenetic substance (Scharloo, 1987). The amount of Make in turn depends on the various environmental or genetic factors that are known to influence the trait. The GEPM is the mathematical relationship that describes what phenotypic values result from given values of Make. The shape of a GEPM determines how variation in Make (small frequency distributions on the abscissa, Fig. 1) translate into variation of the trait (corresponding frequency distributions on the trait axis, Fig. 1). Therefore, a shift of the mean value of Make by some factor not only causes a shift of the mean of the trait, but also a characteristic change in the variance, which reflects the shape of the GEPM.

The shape of the GEPM, depends on the underlying physiological processes. These are unknown in most cases, but it is assumed that differences in the physiology are reflected in differences of the shape of the GEPM. If two factors exert their influence on the trait through the same physiological mechanism, we expect that the variance of the trait scales similarly with its mean regardless which of the factors is involved. This is what Scharloo (1987) found. In his experiments, shifting the mean of the length of the fourth wing vein in the mutant *Hairless* was

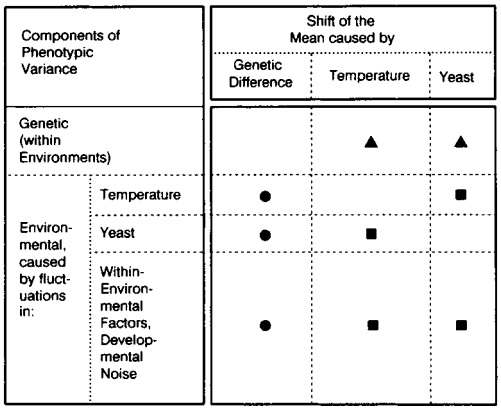

Fig. 2. Sketch of the relationships between means and variation that are examined in this study. The rows correspond to components of phenotypic variance: genetic and environmental. The environmental component is further subdivided into components that are caused by the variation of different factors in the environment as indicated. The columns correspond to causal factors that may shift the mean of a trait, possibly paralleled by a change in the components of variance. A marker in a cell indicates that the relationship between the mean of the trait and the component of variance was examined in this study, where the mean was shifted by the factor appearing in the column heading. Three groups of cases are distinguished by different markers: dots, when the relationship examined was between genetic means and the environmental variance components; triangles, when it was between environmental means and the genetic variance component; and squares, when the means were shifted by a given environmental factor and we observed the effect on an environmental variance component which was caused by another environmental factor.

paralleled by a characteristic change in the variation of the trait, no matter whether the shift was caused by changing the temperature, changing the genetic background by introducing different dominant markers, or by selection. He modelled a physiological mechanism by postulating some morphogenetic substance that induces wing vein formation, and concluded that the experimental manipulations led to variation in this substance. Equivalent changes in the substance caused by different manipulations resulted in the same phenotypic response, because the same mapping described the relationship between the amount of the substance and the length of the vein.

Strictly, similar scaling does not necessarily imply similar mechanisms, although in Scharloo's case the pattern was so characteristic that the hypothesis was strongly supported. However, different scaling does imply different mechanisms. We shall therefore examine how the means of developmental time and dry weight scaled with their variances when shifts were caused by different factors. The design of our experiments allowed us to do this in considerable detail, because we could distinguish between several components of phenotypic variation, and between several factors that cause shifts in the mean (summarized in Fig. 2). We use the GEPM concept to see how each of the components of phenotypic variation scales with the mean if the latter is shifted by either temperature, yeast concentration, or genotypic differences. The analysis points to different modes of developmental and physiological action of these factors and helps identify areas of *Drosophila* physiology and development to examine directly in the future.

Materials and methods

Flies

Isofemale lines were established from single inseminated *Drosophila melanogaster* females that were caught at several sites in and around Basel, Switzerland, in late summer 1986. About one year later, a number of stocks were made homozygous for the first three chromosomes (about 97% of the genome), using chromosome balancers after a protocol similar to that of Craymer (1984).

Experiments

Six of the homozygous stocks (originating from different females) were used to repeatedly produce the same heterozygous progeny that could be tested under a set of 9 environmental conditions defined by 3 food levels (5, 10 and 40 g dried yeast per kg medium) at three temperatures (22, 25 and 28° C). Only one environment was handled at a time.

The 6 stocks were crossed in all 36 possible ways, resulting in a full diallel cross (Mather and Jinks, 1982). For the present analysis, only the 30 heterozygous F1 families were used, and the data within each of the 15 pairs of reciprocal crosses were pooled. The crosses were replicated within each environment in three random-

ized blocks. Each of the 30 heterozygous F1 families was represented by one vial in each block. The vials were randomly positioned in a device that sampled emerging flies at regular intervals to determine their developmental time (described in detail in Stearns et al., 1987). Three such devices (each representing one block) were placed on separate shelves within the same climate chamber that controlled temperature to an accuracy of $\pm 0.1^{\circ}$ C and humidity to $80 \pm 10\%$.

One treatment was defined by its regime of temperature and yeast concentration and consisted of three main steps. First, the parental flies were raised under standardized conditions. Second, the crosses were set up by combining 12–15 females and eight males per cross and block. Egg laying substrate was exposed to the mated flies for 4 hours. Third, the day after egg laying 12 larvae per replicate were transferred to vials containing 2 ml of the experimental medium. Emerging flies were sampled every 6 hours until all had emerged. They were shock-frozen and the time interval of emergence recorded. Later they were dried for 3 hours at 70° C and immediately weighed to the nearest 0.01 mg. A detailed description of the derivation of stocks, stock keeping and experimental procedures is given in the accompanying paper (Gebhardt and Stearns, 1993).

Analysis

To compare the physiological action of different environmental and genetic factors, we compared how the different components of phenotypic variation listed in Fig. 2 on the left were affected when the mean was shifted by one of the factors appearing in the column headings of Fig. 2. Three groups of comparisons can be distinguished. In the first group, the means are genetic values (dots in Fig. 2). They were calculated as the means of the individual data points for the 15 heterozygous genotypes. The variation was caused by unknown within-environmental factors, or also by temperature or yeast concentration, according to whether the genetic values were calculated for each environment separately, or after the data had been pooled over the temperature or yeast gradient. The relationship is then expressed as the correlation between the genetic values and the corresponding environmental standard deviation.

In the second group (triangles in Fig. 2), the means are averages of genetic values ("environmental values", since an environment is characterized by the genotypes measured in it, Falconer 1989, p. 136). The variation is genetic. It is the variance among the genetic values from which the environmental value is calculated. The relationship is then expressed as the correlation between the environmental values and the corresponding genetic standard deviations.

In the third group (squares in Fig. 2), we analyzed how one environmental factor that causes shifts of the means affects the component of environmental standard deviation that is caused by other environmental factors in the experient. To give an example, if we asked how a shift in a mean that was caused by a temperature difference was associated with a change in the component of environmental standard deviation that was caused by variation in yeast concentration, we pooled

for each genotype the individual data for all yeast concentrations within the different temperatures. This resulted in clusters of 15 means and standard deviations for each temperature that could be examined graphically.

Results

The phenotypic responses of developmental time and dry weight to temperature and yeast concentration is described in detail in the accompanying paper (Gebhardt and Stearns, 1993). Briefly, increasing the temperature shortened developmental time and caused weight at eclosion to decrease. Increasing the amount of yeast shortened developmental time and caused weight at eclosion to increase. There were differences in both weight and developmental time among the crosses. We could therefore examine how the various components of phenotypic variation were related to differences in the means caused by one of these factors.

First, we examine the relationship between means and standard deviations if the means are shifted due to genetic factors. The environmental standard deviations of developmental time scaled positively with the means when all environments were pooled (Tab. 1, first row in the panel for each sex), and when the data were pooled over the yeast gradient (within temperatures; next three rows in Tab. 1). No statistically significant relationships were found when the data were pooled over the temperature gradient (within yeast levels; last three rows). Within each environment, the correlations were weak ($r = 0.26$ on average) and only 2 of the 18 correlations (2 sexes times 9 environments) were statistically significant (data not shown). It can be concluded that gene substitutions that increase developmental time have almost no effect on the environmental variation of developmental time if

Table 1. Correlations between environmental standard deviations and genotypic means for developmental time. The means are for individual data within 15 heterozygous genotypes, with data from different ranges of environmental conditions pooled as indicated. A shift of the mean is therefore caused by genotypic substitutions. The standard deviations represent environmental variation of the trait that is caused by the environmental factors over whose range the data have been pooled (plus within-environmental factors). Statistical significances are according to the symbols: ***($P < 0.001$), **($P < 0.01$), *($P < 0.05$) and (*) ($P = 0.07$).

Temperature	Yeast	Correlation	
		males	females
22–28	5–40	0.89***	0.81***
22	5–40	0.71**	0.11
25	5–40	0.62*	0.48 (*)
28	5–40	0.76***	0.80***
22–28	5	0.21	0.27
22–28	10	0.16	0.37
22–28	40	0.11	0.35

the variation is caused by within-environmental effects or temperature, but increase the variation if it is caused by variation in the yeast concentration. For dry weight, we found no consistent correlations between means and environmental standard deviations (in the analogue to Table 1 for dry weight only one of the 14 correlations was significant, and the correlations showed no pattern).

Next we examined the relationship between means and standard deviations if the means were shifted because an environmental factor was changed (Fig. 3). The relationship can be examined using all 9 environmental values, or only the three within a fixed level of one of the environmental gradients (e.g. the three yeast levels at 28° C). For developmental time, the genetic standard deviations tended to scale positively with the means (Fig. 3a), and for dry weight negatively (Fig. 3b). However, the relationships are only weak and not statistically significant (develop-

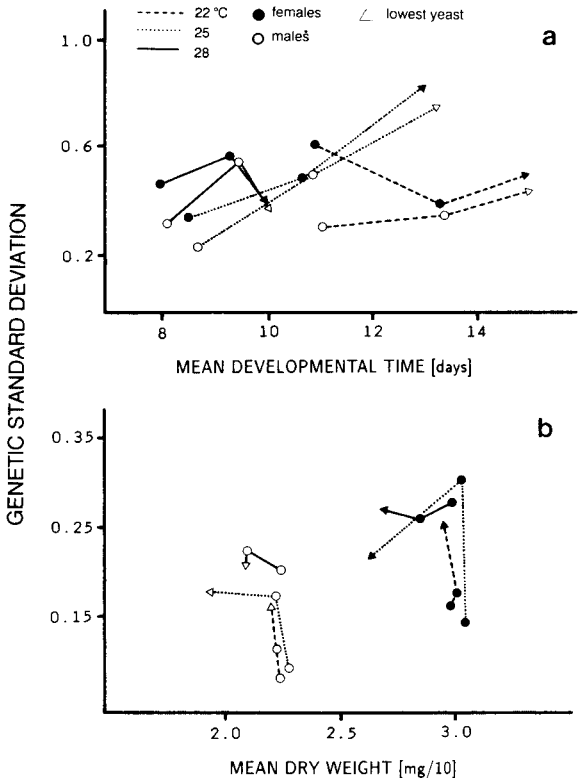

Fig. 3. Relationship between genetic standard deviations and environmental values (Falconer, 1989) for developmental time (**a**) and dry weight (**b**) in each environment. The *y*-axis represents the standard deviations among genetic values, the *x*-axis the means of the genetic values. Each point represents one environment. To facilitate the interpretation, lines are drawn that connect points corresponding to environments with same temperature along the yeast gradient. Triangles mark the lower end of the yeast gradient (5 g/kg).

mental time: $r = 0.39$ for males, 0.31 for females; dry weight: $r = -0.55$ for males, -0.22 for females; all 9 environmental values used). If only three environmental values are used at a time (at a fixed level of one of the factors), this general pattern still holds (for example, the correlations were always negative for male dry weight, albeit never significant), but the figure shows that there was much scatter in the data. A conservative conclusion is that the relationships between environmental values and genetic standard deviations (this paragraph, Fig. 3) are not the same as the relationships between genotypic values and environmental standard deviations (previous paragraph, Tab. 1).

We also asked how a shift in the mean of a trait caused by a shift of one environmental factor (say temperature) influences the component of environmental variation of the trait that is due to variation in another environmental factor (say yeast concentration). In Fig. 4, environmental effects can be assessed by comparing the positions of whole clusters: equal sizes of symbols indicate equal yeast concentrations and show the effect of temperature differences, equal shape of symbols indicate equal temperatures and show the effect of differences of yeast concentration. Three observations can be made. First, comparing the positions of clusters corresponding to different temperatures shows that temperature differences caused differences of the means, but not of the standard deviations. Since these standard deviations were measured within each environment, we can conclude that the within-cross means and environmental standard deviations were not related if the standard deviation were caused by within-environmental factors. Second, within-cross means and environmental standard deviations were positively related, if the mean was shifted by a change in yeast concentration (compare clusters within temperature). Third, within-environmental standard deviations did not scale consistently with the means for each cross within environments, as there was no consistent relationship within each single cluster. This agrees with the finding

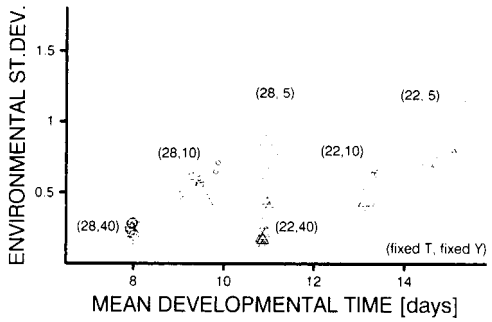

Fig. 4. Relationship between environmental standard deviations and genetic values for developmental time. The y-axis represents the within-genotype standard deviations from each separate environment, the x-axis the genetic values from each separate environment. The points from each environment cluster together and are separated by different symbols (circles: 28° C, triangles: 22° C, large, medium, small symbols: 40, 10, 5 g yeast/kg). Since females and males were similar, only male data are shown. Data from the intermediate temperature are left out for clarity.

reported above that there were almost no significant correlations between genetic means and within-environmental standard deviations.

The scatterplots in Fig. 5 are similarly constructed, but this time the within-cross means and standard deviations are calculated by pooling the data over one of the environmental gradients (the yeast gradient in panel a, the temperature gradient in panels b and c). By comparing the positions of clusters the following can be observed. Mean developmental time was positively related to the component of environmental variation caused by variation of yeast concentration if the mean was shifted by reducing the temperature from 28° C to 25° C, but not from 25° C to 22° C. (Fig. 5a). Mean developmental time was positively related to the temperature-caused component of environmental variation if the means were shifted by yeast concentration (Fig. 5b). In contrast, mean dry weight was negatively related to the temperature-caused component of environmental variation (Fig. 5c).

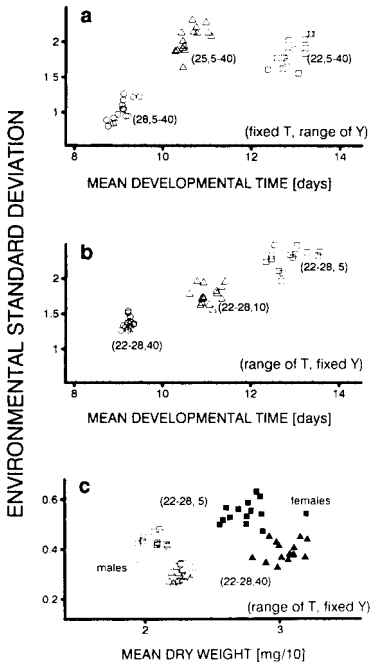

Fig. 5. Relationship between environmental standard deviations and genetic means for developmental time (**a** and **b**) and dry weight (**c**). The difference from Fig. 4 is that the data are not calculated from each environment separately, but by pooling across one of the two environmental gradients (within genotypes): over yeast concentrations on panel **a** (clusters labelled by "(fixed T, range of Y)"), and over temperatures on the lower panel (clusters labelled by "(range of T, fixed Y)"). Therefore, the comparison of the positions of clusters on panel **a** shows the overall effect of temperature shifts on means and standard deviations, and on panels **b** and **c** the effect of shifts of the yeast concentration. As in Fig. 4, only male data are shown for developmental time on panels **a** and **b**. On panel **c**, open symbols indicate male data, filled symbols female data. The shapes of the symbols correspond to the different ranges of environments, as indicated by the values in parentheses next to each cluster.

Sex, a kind of genetic factor, also affected both means and standard deviations. This effect was too small for developmental time to be examined here, but was considerable for dry weight. Females were heavier than males, and it turns out that all components of phenotypic variation of dry weight scaled positively with this difference in the mean. This is shown for the genetic component in Fig. 3b (comparing corresponding open and filled symbols). It is true for the within-environmental component: in only 14 of the 225 comparisons (15 genotypes in 9 environments = 225) were females not more variable than males. The mean variance ratio of females to males was 1.78. It is also true for the environmental component due to variation of yeast concentration (in all of the $15 \times 3 = 45$ comparisons; the mean variance ratio was 1.82) and for the environmental component that is due to variation of temperature (in 43 of the 45 comparisons; the mean variance ratio was 1.72). For the latter component the higher environmental standard deviation of females can also be seen in Fig. 5c.

All these relationships are summarized in Table 2, which is constructed like Fig. 2. The signs indicate how variation of a particular factor (column headings) affect the different components of phenotypic variation of a trait that are caused by variation of another factor (rows in the table).

Table 2. Summary of the correlations between means and standard deviations. The entries indicate the sign of the correlation of a mean with the component of phenotypic standard deviation that is caused by the factor indicated in the row descriptions (left-most column), when the variation in the mean of the trait is due to a shift of the factor indicated in the column headings. For example, the positive signs in the column labelled "Genetic Diff." for developmental time indicates that if the mean was increased by genetic differences, the component of phenotypic standard deviation that was caused by yeast variation also was increased. Parentheses indicate that the correlation was only a weak trend. Because sex differences were too small for developmental time, they were not examined and have no column entry.

Component of Phenotypic Standard Dev.	Developmental time			Dry weight			
	Genetic Diff.	Temp. Shift	Yeast Shift	Genetic Diff.	Temp. Shift	Yeast Shift	Sex Diff.
Genetic (within Environments)		(−)	(+)		(−)	(−)	+
Environmental (due to Temperature)	0		+	0		−	+
Environmental (due to Yeast)	+	(+)		0	(−)		+
Within-environmental	0	0	+	0	0	0	+

Discussion

Rendel (1967) and Scharloo (1987) summarized experiments in *Drosophila* that suggested that simple mapping functions can be used to describe how different genetic or environmental factors produce phenotypic variability. It is important to note that mutant characters were under study in their experiments, and that the mapping functions that were found by the analysis of the relationship between mean and variance were specific for particular mutations. Two mutations that cause variation in the length of the fourth wing vein, ci^D and *Hairless*, were associated with different mapping functions. This shows that the shape of the GEPMs is subject to genetic variation. However, having defined the major gene that controls the character (e.g. *Hairless*), the relationship between mean and variance supported a model where different factors (genetic background as changed by directional selection or chromosome substitution, and environmental variables) affected development in a similar way.

A different picture emerged in our experiments (Tab. 2). Genetic differences were not associated with discernible trends in the standard deviation of dry weight, while for developmental time, whether a trend was observed or not depended on the environmental factor that caused the variation. This agrees with the result of many reports, mostly on plants, that mean performance of genotypes and their sensitivity to environmental change are largely independent traits (Boughey and Jinks, 1978; Schlichting, 1986). One may conclude that the differences among genotypes in our study are more comparable to the difference between different mutations (*Hairless* or ci^D) than to the differences in the genetic background produced by selection in Scharloo's experiments. On the other hand, an environmental shift had a clear effect on both genetic and environmental standard deviations, with the sign depending on the factor causing it.

Two observations thus indicate that, for these two life-history traits quite different processes are affected when shifts in mean performance are accomplished by different causal factors. First, contrary to the results with wing vein length, but similar to many plant experiments, the overall relationships between mean and standard deviation was different for different gradients. Second, a given source of a change in the mean had different effects on different components of variation. This suggests that the factors causing the observed variation act at different sites within the epigenetic apparatus. A good example is developmental time compared among different temperatures. If one pools all the data within each temperature, the observed phenotypic variation is due to variation in yeast concentration, unknown within-environmental factors or developmental noise, and differences among genotypes. Each of these sources of variation must act at different sites because they interacted differently with temperature (different signs in the column for temperature shifts in Table 2).

The most clear-cut case (although also the least surprising) can be seen in the relationship between mean and standard deviation of dry weight when females and males are compared. Females are heavier than males and are also more variable, although the general relationship between mean and standard deviation is negative

for this trait if a relationship exists (Tab. 2). Clearly the substitution of an X for a Y chromosome affects the developmental system quite differently from any other factor. This might sound somewhat trite at first, because it is clear that males do not develop ovaries if they get more to eat. However, it is less obvious that increasing weight by increasing the organs of males should not also increase variability rather than decrease it, as it does.

The concept of GEPMs has been valuable because it suggests an analysis that points towards mechanisms of development, as in the example of wing vein length. An explanation for the clear-cut response patterns found by Scharloo – as contrasted to our observations – may be that a small segment in the series of developmental steps that produce a morphological character was sensitized by the mutants involved. For other cases, such as ours, the analysis shows that models and experiments on physiology and development must incorporate different roles for different causal agents. The reasons for the different roles can be manifold. They must be analyzed with appropriate experiments that differentiate between the separate steps in development. For example, Pantelouris (1957) demonstrated that the response of thorax size and wing length to temperature shifts during *Drosophila* development depended on the pattern of the shifts because the sensitive developmental steps occurred at different times during development for the two characters. Complex phenomena can also arise with size-related characters because size is a function of cell size and cell number in multicellular organisms. Robertson (1959a, b) addressed this issue in a series of experiments with *Drosophila.* Genetic and environmental factors had different effects on the variation of wing size, wing cell number and cell size. Furthermore, different response patterns were obtained when overall wing size was manipulated either by direct selection or by selection on cell size, suggesting that different genetic factors were targeted, acting either on cell growth (and apparently involving complex epistatic interactions) or on cell number (in a more direct, additive way).

Such complex mechanisms may be the rule (Parsons, 1961), and evolutionary models should consider that different environmental factors can have different effects on the composition of phenotypic variance. If genetic and environmental variance components of the trait are changed in parallel, then environmental heterogeneity may have only small effects on evolutionary rates, because the heritability changes little. If the two components of variance are inversely affected, then evolutionary rates may be strongly affected, because the heritability will change in either direction. Both can happen, as is shown by the example of developmental time when either temperature or food resources were the predominantly unstable factors in the environment (Tab. 2).

Lastly, we want to comment on the uncritical computation of coefficients of variation, the ratio between standard deviations and means. Often they are used to "standardize" the variation when the means are different among groups of comparison. However, our analysis shows that such procedure has to be justified, as it is not trivial how standard deviations scale with means. Why should there be a linear relationship between standard deviations and means? In fact, the very phenomenon of scaling is perhaps the starting point of a whole area of research.

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