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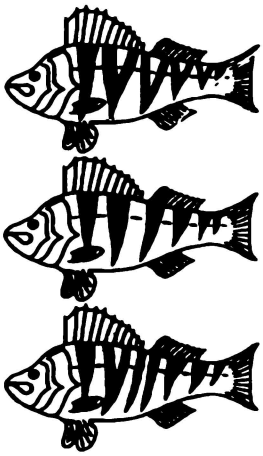

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Analysis of the genotype-environment interaction in natural populations

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The discovery of a genotype-environment interaction in a natural population does not mean that genotypic and ecological factors are important at the same time. The importance of genotypic factors is indicated by the effect of families, clones. The importance of ecological factors is indicated by the effect of different variants of the environmental conditions. The genotype-environment interaction is a 'third power', which cannot be reduced to the formula "genotype + environment". The phenomenon of genotype-environment interaction cannot be divided into separately estimatable parts — the genotypic and ecological components.

1. Introduction

The variability of quantitative characters in natural populations is undoubtedly of interest. Far be it for us to neglect other approaches, such as isozymes, but let us note some important features of quantitative characters. First, those characters are generally quantitative which determine the fitness of individuals. Second, the differences between the values of quantitative characters for the individuals in natural populations are apparently conditioned by a multitude of genes. Therefore, a few quantitative characters reflect a considerable portion of the genome. And that portion is representative of the genetic heterogeneity of populations as a whole rather than separate loci. Third, the manifestation of quantitative characters is normally strongly dependent on environmental conditions and genetic background; therefore it is possible to reveal not only specific genotypic and ecological effects, but also the interaction of genotype and environment. Last, the investigation of quantitative characters, chosen reasonably, is technically simple, and practical for any species of plant or animal. The last circumstance is extremely important. As N. V. Timofeeff-Ressovsky repeatedly emphasized, in order to make progress, however small, in the development of population biology and microevolutionary theory, the population-genetic research should involve a great many species of plants and animals differing in their systematic position, biological peculiarities, and place in the biogeocoenotic structure.

2. Revealing and quantitatively estimating the genotype-environment interaction

Previous investigations did not consider quantitative estimation of the genotype-environment interaction (Clausen et al. 1940, Parsons 1977, Giesel et al. 1982, Gupta & Lewontin 1982). For that purpose, we offer a rather versatile technique, applicable to virtually any species of plant or animal from nature. Let us consider this experimental procedure with an example from our work on *Drosophila* (Glotov & Tarakanov 1983).

Naturally fertilized females of *Drosophila melanogaster* were caught in the gardens of Ubinskaya village, in the north-west Caucasus, and each fly was separately and successively placed on media differing in nutritiousness — rich, normal, and poor. Eight progeny from each female parent on each medium were taken to measure quantitative morphological characters. Thus, we obtained a factorial design for a two-way analysis of variance, with eight replications in each cell (orthogonal complex). Then the total variability in each character was decomposed into the following components: inter-environmental (ecological), inter-family (genotypic), family-environment (genotype-environment) interaction, and the residual, uncontrollable variability. Of course what we evaluate in this simple scheme is only the lower bound of genotypic variability because, as a consequence of splitting, the differences between the individuals within a family include the genotypic component as well.

What does genotype-environment interaction mean here? Let us refer to Fig. 1. At the top of the figure, we have the case where the interaction is zero; the population mean values are different for different media (i.e. there is an influence of environment). The mean values for the three families shown in the figure are also different, but the straight line segments P-N and N-R for every family are parallel to the lines passing through the population mean values. At the bottom of the figure one can see differences between the media and between the families, but the segments for two of the three families are not parallel to the lines passing through the population mean values. The genotype-environment interaction means that the segments for at least some

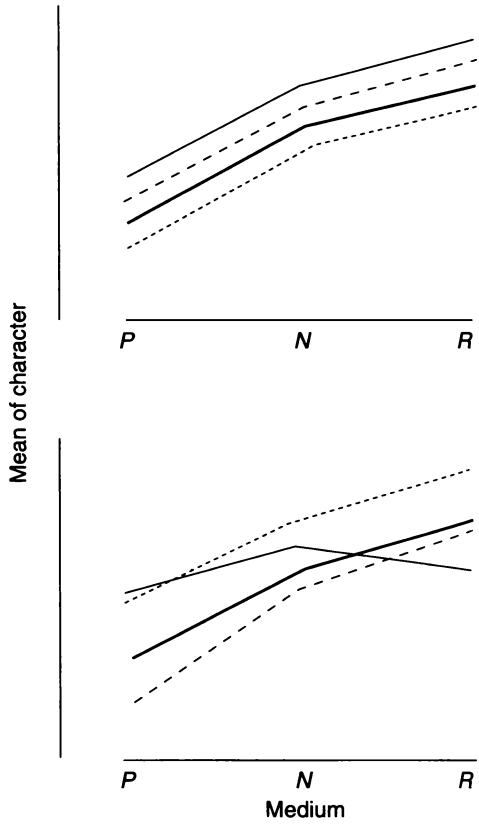

Fig. 1. Absence (top) and presence (bottom) of the genotype-environment interaction. The bold line passes through the population averages; the other lines pass through the averages for separate families.

of the families are not parallel to the lines passing through the population mean values. The degree of non-parallelism serves as a measure of interaction.

We examined the progeny of 169 females from a natural population. Table 1 shows the contribution of various factors to the total variability of the characters. One can see that both genotypic effects and the genotype-environment interaction are observed in all of the characters investigated. Note that the magnitudes of the contributions are nearly equal. They are systematically reproduced in repeat experiments and are not related to the spatial structure of populations. Just like the variance of separate characters, the covariance of all possible pairs of characters can be decomposed into components.

Table 1. Percentage contribution of various factors to the variation of quantitative characters in *Drosophila*.

	Medium	Family	Interaction	Residual
Wing length	80.4	4.0	6.4	9.2
Femur length	86.5	2.4	4.4	6.7
Number of sternopleural bristles	39.9	12.3	3.3	44.5
Number of abdominal bristles	44.0	11.1	7.9	37.0
Number of arista side branches	27.3	9.0	3.7	60.0
Pairs of characters (on the average)	85.5	2.0	5.1	7.5

The structure of all covariances proved to be surprisingly uniform, while the structures of different variances were essentially different, so we have averaged the data presented in Table 1 over all covariances.

3. Proportion of families responsible for the genotype-environment interaction

It is very important to answer the question: How large is the fraction of families that determines the genotype-environment interaction in a population? The genotypic structure of a population will be quite different depending on whether the interaction effect is due to particular individuals, or comes about as a massive phenomenon based on the genetic heterogeneity of populations. To answer the question, we use the following procedure. We single out the contributions of separate families to the interaction sum of squares and rank them. The family that makes the greatest contribution is eliminated from the analysis. The remaining families are subjected to analysis of variance anew. The contributions of separate families to the interaction are determined again, and again, the family making the greatest contribution is eliminated. The procedure is continued until the interaction ceases to be statistically significant at a level of five percent. Note that this method allows one only to establish the lower bound for the proportion of families that bring about the genotype-environment interaction; statistical conclusions depend on the sample size, and our procedure is based on the successive reduction of the sample. Table 2 demonstrates that the genotype-environment interaction is really brought about by the genetic heterogeneity of the population.

Table 2. Proportion of families in the population making a contribution to the genotype-environment interaction.

Characters	Proportion (%)
Wing length	58.0
Femur length	56.8
Number of sternopleural bristles	8.3
Number of abdominal bristles	24.9
Number of arista side branches	7.7
At least in one character	74.6

4. Types of reaction norms for genotypes in a population

The investigation of the genotype-environment interaction, if it does not give an idea of the distribution of reaction norms for the genotype in the population, eventually allows at least some of the types of reaction norms to be distinguished. The approach described below was put forward by V. V. Tarakanov. Among the families responsible for the genotype-environment interaction, the presence of two types of families can be expected:

1) stable families, showing relatively little decrease in the value of the character on the poor medium and relatively little increase in the value of the character on the rich medium, and
2) unstable families, showing a greater decrease in the value of the character on the poor medium and a greater increase in the value of the character on the rich medium. As a measure of stability of the family, one may use the standard deviation for the mean val-

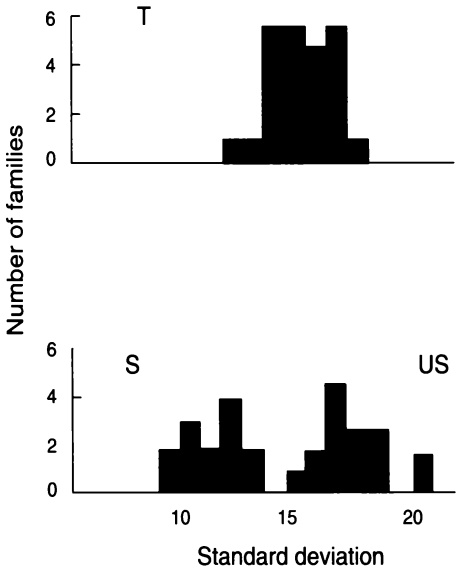

Fig. 2. Distribution of the standard deviation "among the media" for the typical (T), stable (S), and unstable (US) families in respect of the character of wing length.

ues for the given family for the different media. An example of such analysis for one of the samples, by the character of wing length, is shown in Fig. 2. The distribution at the top of the figure is representative of a typical family (T) making no contribution to the interaction. The distribution at the bottom of the figure is of two apices: the stable (S) families are separated from the unstable (US) ones by a clearcut hiatus. Identical graphs were obtained for other samples. Averaged over the whole material, the proportion of typical families amounts to 42.3%, stable families 23.8%, and unstable families 33.9%.

The diversity of the types of reaction of individuals in a population can be attributed to the existence of certain alleles that influence the norm of reaction; but most likely, it is due to certain combinations of genes arising during recombination. An answer to this question can be found in a special experiment. It is necessary to first assess the type of reaction of the corresponding parents, then to derive population boxes separately from typical, stable, or unstable individuals and a mixture thereof, and then observe the dynamics of the number of individuals, and fitness and variability of quantitative characters in those experimental populations. It would be of interest to combine such an investigation with a simultaneous study of the set of enzyme loci. We believe that there is a great future in the analysis of the types of reaction norms with the total control over the entire genome of *Drosophila* by means of the technique similar to that of Gershenzon (1941).

Lastly, is the reaction norm of the genotype specific to the factor (nutritiousness of the medium) acting on a general character, independently of the contrasting backgrounds (nutritiousness of the medium, thermal action, competition with other species, etc.)? Anyway, using the density of *Drosophila* larvae as a differentiating ecological factor, Tarakanov et al. (1988) obtained a similar structure of variability.

5. Necessity of combining field and laboratory research

Peculiar to the approach offered here — the analysis of related individuals against ecologically contrasting backgrounds — is the combination of field and laboratory investigations. The individuals are taken directly from the natural population where they have lived, and freely interbred. (It goes without saying that various other investigations in natural populations are also carried out in this way — e.g., for *Drosophila* see Glotov et al. 1986; for the works with *Festuca* mentioned below, see Gritzenko et al. 1984). Then, the progeny of the individuals from nature (or clones) are tested under laboratory conditions for no longer than one generation.

There are data suggesting that the maintenance of panmictic populations of *Drosophila*, however large, for a long time under laboratory conditions reduces variability in characters and fitness, apparently because of stabilizing natural selection, not controlled by the experimenter (Hartl & Jungen 1979). This has recently been observed by Imasheva et al. (1986) for a complex of quantitative characters of the wing. In the experiments performed jointly by V. V. Tishkin

and ourselves, the fitness of *Drosophila* was compared using the compound-2 lines (Jungen & Hartl 1979) in the following variants of breeding a pair of wild-type individuals and four pairs of compound-2 individuals per vial:

A) Individuals caught directly in the natural population from Ubinskaya (the north-west Caucasus) (Tishkin & Glotov 1983).
B) Box population derived from individuals out of a natural population. The population was derived from about three thousand native flies, the number of individuals ranged from 6 to 9 thousand. The fitness test was conducted after about 30 generations.
C) Inbred lines. *Drosophila* lines were derived from 320 naturally-fertilized females. The lines were maintained by inbreeding, each subsequent generation being derived from a single female. The estimation of the fitness of pairs of individuals was performed after 25 generations in two repeat experiments with 101 and 98 lines.
D) Box population from a mixture of inbred lines. After 25 generations of inbreeding, a pair of individuals was taken out of each of the 186 lines that had survived by that time and put into a population box. The fitness was tested after 7 generations of box breeding.

One can see (Table 3) that the fitness of the box population derived from native individuals is considerably reduced, coming down to the same level as that reached from below by the fitness of the other box population, derived from the mixture of inbred lines after the 'resynthesis' of the population variety.

It appears important, therefore, to maintain and test individuals of a native population (or their descendants) in laboratory conditions within a limited space of time.

6. Some methodological questions

Two questions arise in connection with the assessment of the genotype-environment interaction according to the above procedure. First, how are the components of variance affected by the difference between the developmental conditions of the parents (i.e. ecological after-effect)? And second, what is the influence of the differences between the conditions in different vials with the same medium (microfluctuations of environment)? In cooperation with I. V. Jacobson, we studied a model system — 40 hybrid combinations of F_1s between pairs of different laboratory lines of *Drosophila*, where the various hybrids represent analogs of individual females from nature. A scheme of the experiment is shown in Fig. 3. Each hybrid combination of F_1s was obtained on the poor and the rich medium. Each F_1s individual produces F_2s on the poor, normal, and rich medium. Two females of each F_1s variant were taken from each medium. The examination of the structure of variability of characters (wing length and number of sternopleural bristles) in the F_2s showed that the differences caused by the ecological after-effect and microfluctuations of environment made a total of approximately 8 percent of the variance for wing length and 1 percent of the variance for bristle number (Table 4). The proportion between the genotypic component of variance and the component due to the genotype-environment interaction was close to that observed with the same material in the two-way ANOVA (disregarding the ecological after-effect and microfluctuations of the environment), indicating its effectiveness. The structure of variability of characters as given by the two-way analysis of variance is close to that observed for the analysis of individuals from a natural popula-

Table 3. Fitness of *Drosophila* for different types of breeding (Tishkin & Glotov 1983).

		Parent pairs	Descendants total	Descendants normal	Fitness
Native population	(A)	124	12.032	10.965	163.8
Box population from native individuals	(B)	89	5.743	3.826	31.9
Inbred lines	(C)	101	5.200	2.005	10.1
		98	4.017	1.744	12.3
Box population from a mixture of inbred lines	(D)	87	4.662	2.845	25.1

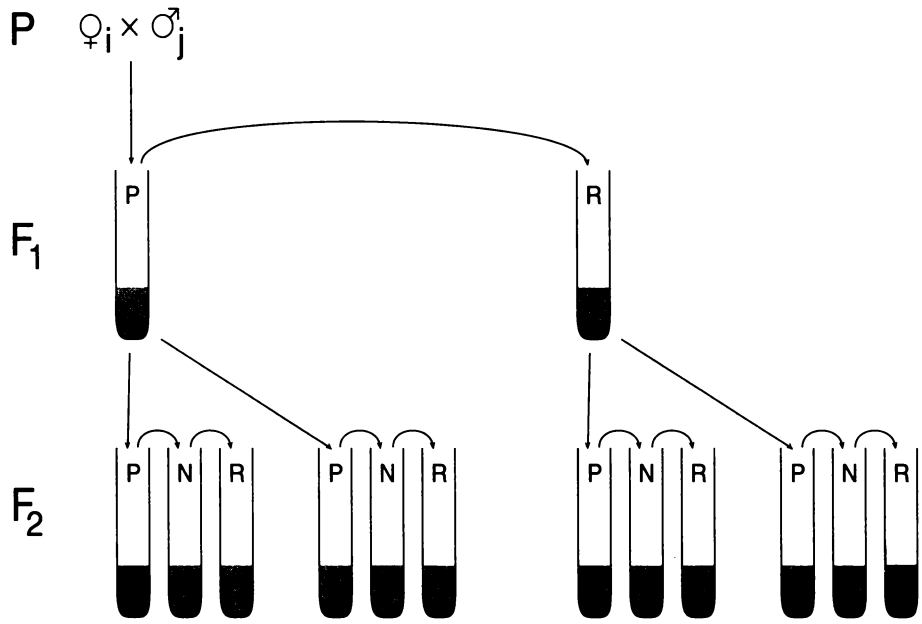

Fig. 3. Scheme for the experiment to estimate the influence of ecological after-effect and microfluctuations of the environment on the structure of variation of quantitative characteristics in *Drosophila*. For an explanation, see the text.

Table 4. Percentage contribution of various factors for the four-factor scheme of dispersion analysis (Jacobson & Glotov).

Source of variation		Wing length	Number of setae
Medium F_1	(A)	1.0	1.2
Medium F_2	(B)	58.0	22.0
Hybrids of F_1	(C)	5.1	29.5
Test-tubes	(D)	3.8	0.0
Interactions:	AB	0.5	0.0
	AC	2.7	0.0
	BC	4.1	1.8
Residual		24.8	45.5
A + D + AD + AC		8.0	1.2

tion, i.e., the model gives a sufficiently precise picture of the behaviour of the natural population.

Another methodological aspect concerns the statistical procedure. Do the data obtained in that sort of experiment conform to the model of a two-way analysis of variance? And if not, how informative are the inferences made using analysis of variance? We carried out a special investigation with a view to answer those questions (Glotov & Rachman 1989). It turned out that any one of the assumptions, without exception, underlying the theory of the mixed model analysis of variance fails to be fulfilled for the data available. However, it can be demonstrated using the 'discordance criterion' proposed by M. I. Rachman that, at least for an orthogonal complex (as in this instance), the conclusions drawn from analysis of variance are quite reliable, as a matter of fact.

7. Dependence of the manifestation of the genotype-environment interaction on the amplitude of deviation of ecological conditions from the optimum

This dependence was established in the work where we analysed the experimental data of I. N. Dregolskaya (Institute of Cytology, USSR

Academy of Sciences, St. Petersburg) on the change in the heat resistance of *Hydra oligactis* Pall. in response to rises and falls in the temperature of the environment by different amplitudes. The polyps were caught out of a pond near St. Petersburg, and the clones derived from separate individuals were shifted to a reduced (6, 10, 15, 18°C) temperature (cold acclimation) or an elevated (23, 25, 27°C) temperature (warm acclimation) for 24 hours for thermal acclimation. After 24 hours, the hydra were exposed to test temperature, 35°C. The criterion of heat resistance was the logarithm of survival time for an individual at the test temperature (Ushakov et al. 1968, Dregolskaya 1977).

We performed the data analysis in accordance with the assigned task in the following manner. (Let us consider the course of reasoning on the example of the cold acclimation). The living conditions become less suitable for the hydra as the temperature of the environment decreases. It is hard to say how far things get different, but this much is certain: the ecological conditions at, say, 18°C depart less from those at 21°C than do those at 15°C. Thus, the nearest ecological differences are the case with the pair 21–18°C, farther ones with the pair 21–15°C, still farther ones with the pair 21–10°C, and eventually the farthest ones in that experiment with the pair 21–6°C. So, it is possible to analyze the variability of heat resistance for the clones of the same hydra accompanying the increase in the deviation from the ecological optimum by comparing the pairs 21–18°C, 21–15°C, etc. The variability of heat resistance in the warm acclimation, accompanying the increase in the contrast of the ecological conditions in the series 21–23°C, 21–25°C, 21–27°C, can be analysed in a similar way.

The increase in the contribution of the clone-conditions (genotype-environment) interaction with an increase in the deviation from the ecological optimum is shown in Fig. 4. However, admitting that the genotype-environment interaction is more fully disclosed in 'hard' ecological conditions, the possibility cannot be excluded that the relatively 'soft' ecological conditions may exist for a certain species of organism and a certain character which would make the interaction show itself as fully as possible.

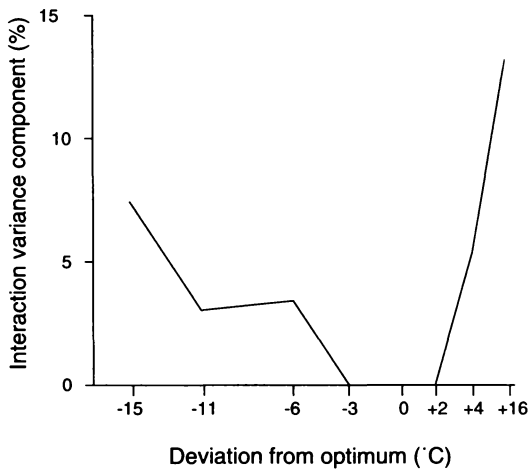

Fig. 4. Part of the genotype-environment interaction in the general variability of hydra at different magnitudes of deviation from the optimum temperature of development.

8. The possibility of using the express test

Estimation of the genotype-environment interaction by means of analysing characters in relatives against ecologically contrasting backgrounds cannot be carried out for long-lived species in full measure. It is necessary to explore the possibility of using the express test. This was done for *Festuca woronowii* Hack. in Daghestan populations (Glotov & Gritzenko 1983).

The plan of the experiment was implemented with the material of the Gunib population. The seeds of every one of 37 parent plants were divided into three portions. In one variant of the experiment, the seeds were germinated in a 0.001% solution of gibberelin, which produced a stimulant effect. In another variant, a 0.5% solution of ammonium nitrate was used as an inhibitor. In the third variant (control experiment), the seeds were germinated in tap water. The character under investigation was the maximum length of the germ. The duration of the experiment was 45 days.

The following structure was found for the general variability: the contribution due to the

germination conditions was 84.1%, families 7.7%, and the family-conditions interaction 4.1%. Similarly, experiments were conducted with various clones of *Festuca*. These were grown on the northern and southern microslopes of the Gunib plateau. Family crops of *Festuca* were grown in Gunib and Moscow. The incomparably less laborious express test also proved to differentiate the ecogenetic structure of the population much better.

9. Ecogenetic structure of a population in a polluted environment

The express test described herein was used by L. F. Semerikov (Institute of Ecology of Plants and Animals, Urals Division, Russian Academy of Sciences) to analyze three populations of *Typhoides arundinacea* L. from West Siberia. These populations differ in the degree of pollution of their territory with petroleum. *Typhoides arundinacea* is a perennial rhizomatous grass. The character under investigation was maximum length of the germ. The structure of population variability (Table 5) under light pollution is no different from that described above for *Drosophila, Hydra*, and *Festuca*. The interaction disappears (i.e is not observed at a significance level of 1%) in the case of moderate pollution of the territory with petroleum. As for heavy pollution, the inter-family (genotypic) variability also fails to be observed. The simplest explanation of those results is to admit the action of intense natural selection, reducing the genetic diversity of the population. Under moderate pollution, those individuals are eliminated which differ in reaction norms, and under heavy pollution, so are the individuals differing from the mean population standard.

Table 5. Effect of petroleum pollution on variation of germ length in *Typhoides arundinacea* (L. F. Semerikov).

Pollution	Conditions	Family	Interaction	Residual
Light	59.6	8.3	15.0	17.1
Moderate	69.4	8.0	0.0	22.9
Heavy	61.6	0.0	0.0	38.4

There exists yet another explanation, which seems to us more plausible. Plants are characterized by a very high plasticity (including changes in the values of various quantitative characteristics), an adaptation for sudden changes of environment. Perhaps, different genotypes simply do not exhibit any differences under those conditions, displaying similar values of the characters and similar reaction norms.

How general is the picture of population variability observed by L. F. Semerikov under conditions of petroleum pollution? And which of the mechanisms (or combination of these) is actually operating? These questions are presently an object of our investigations with white clover (*Trifolium repens* L.).

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