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ESTIMATION OF GENETIC HETEROGENEITY IN NATURAL
POPULATIONS: QUANTITATIVE TRAITS

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An estimation of the genetic heterogeneity of natural populations by quantitative traits is proposed for the purpose of characterizing the genetic potential of the various populations within the entire species distribution range and for finding the populations possessing the greatest ecogenetic diversity. Two possible approaches are examined: 1) separation, from the total phenotypic variation, of paratypic components estimated by repeated measurements of the values of a trait on the metameric organs of a single individual, and 2) a family (clone and so on) analysis in an express test against ecologically-contrasting, extreme backgrounds.

The concept of the genetic heterogeneity of natural populations was formulated by S. S. Chetverikov (1926); this work initiated experimental population genetics (Glotov, 1981). The genetic heterogeneity of populations is evidenced every time two or more alleles are found in a population for one or several loci; this also refers to chromosome structure and number. S. S. Chetverikov predicted and the entire aggregate of data of contemporary population genetics convincingly demonstrates that the natural populations of any species are genetically heterogeneous with respect to all traits and properties. In the graphic expression of N. V. Timofeev-Resovskii, whatever mutations are sought can be found in natural populations.

The genetic heterogeneity of populations can be estimated by studying qualitative morphological traits, lethal effects, and depressed viability, as well as biochemical polymorphism (Timofeev-Resovskii et al., 1973; Lewontin, 1974; Dobzhansky, 1970; Nei, 1975). Each method has its advantages and features limiting its range of application.

In connection with the problems of the preservation and rational utilization of the natural environment, the task arises of estimating the genetic potential of various populations within the limits of the entire species distribution range, of finding the populations possessing the greatest genetic diversity and the greatest adaptation to an environment changing in space and in time. Of promise for solving this task is an investigation of the genetic heterogeneity of natural populations by quantitative traits, ideally based on biometrical genetic (Rokitskii, 1974; Mather and Jinks, 1971). Biometrical genetics has a multitude of rather well-developed schemes for genetic analysis. The main limitation for the application of these methods to the task of testing natural populations is the fact that this task requires the analysis of a massive material. Therefore, at least at the first stages of the investigation prior to the identification of a small number of populations of evident promise, most of the relatively labor-intensive methods, associated primarily with setting up crosses, must be rejected.

From our point of view, two approaches are of greatest interest for estimating the genetic heterogeneity of natural populations by quantitative traits: 1) separation, from the total phenotypic variation, of paratypic components, estimated by repeated measurements of the values of a trait on the metameric organs of a single individual, and 2) a family (clone and so on) analysis in an express test against ecologically-contrasting, extreme backgrounds (Glotov, 1979).

DETERMINATION OF METAMERIC VARIABILITY

An obligatory condition for separating the genotypic component of variation in studying a quantitative trait is the measurement of the values of the trait in individuals whose rela-

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tionships with one another are known (sibs, semisibs, parents—progeny, and so on). At the same time it is assumed that all individuals have undergone development under identical environmental conditions.

The study of metameric variation is essentially a special case in this approach. By metameric variation is meant the parts or organs of a single individual that are multiply (at least twice) repeated. The differences between the values of any trait that are recorded on metameres are determined by the norm of the response of a single genotype to microfluctuations in the environment, to the random variation ("noise") during the repeat realization of metameric structures by a single genotype.

Assume, for example, that the trait of "leaf length" is studied in a natural population of some tree species. In so doing, n leaves are collected from k trees. The sample as a whole, consisting of $k \cdot n$ leaves, characterizes the total phenotypic variation in the population with respect to the given trait. By sampling the variance as a measure of the variation, the total variance σ^2 can be broken up, by performing a hierarchical variance analysis (Scheffé, 1959; Ahrens, 1967), into two components — the variance of the character between different trees σ_b^2 and the variance within the crown of a tree σ_w^2 , $\sigma^2 = \sigma_b^2 + \sigma_w^2$. Insofar as the different trees in the populations are under different environmental conditions, the σ_b^2 component reflects compositely both genotypic and ecological differences. Since the described procedure excludes from the overall variation the paratypic component, the difference between different populations with respect to σ_b^2 will emerge more distinctly.

Of special interest is the study of σ_w^2 for different trees: A statistical analysis will reveal individuals possessing minimal and very great metameric variation. Such an approach proved, for example, very effective in studying the traits of leaves and fruits in Caucasian oaks (Glotov et al., 1975; Glotov and Semerikov, 1978; Semerikov and Glotov, 1979, 1980) and a number of plant species in Dagestan (Magomedmirzaev (ed.), 1975, 1977, 1978, 1981).

The need for studying variation at different levels (within the crown, during different years, etc.) is emphasized by S. A. Mamaev (1972), who used the coefficient of variation for this purpose. A comparison of the coefficients of variation for different populations roughly coincides with the analysis of

$$\sigma_b^2 = \frac{1}{k-1} \sum_{i=1}^k (x_i - x_{..})^2 - \frac{1}{n} \sigma_w^2,$$

where x_i is the mean value of the trait for the i -th tree and $x_{..}$ is the population mean. The first term in the right-hand part is the variance between trees σ_m^2 , estimated by the means for each tree. Thus, the σ_m^2 variance, which is somewhat greater than σ_b^2 , is used in examining the coefficients of variation. If the number of measurements n from each tree is sufficiently large (say, $n \sim 10$), then this difference has virtually no meaning. This approach, however, has another deficiency. The variation is studied separately at different levels, whereas in our formulation all components of the variation are considered simultaneously. One cannot agree with the objection that the within-crown variation cannot be estimated by collecting 5-10 leaves from the crown of each tree. Even if n is small and there are $(n-1)$ degrees of freedom for estimating the within-crown variance, for an aggregate of k trees we estimate this variance by a $k(n-1)$ number of degrees of freedom. For example, if $n = 5$ and $k = 30$, then $30 \cdot 4 = 120$! Furthermore, the 30 obtained variances can be investigated in detail after analyzing their distribution.

Ontogenetic differences between metameric organs may present a complication in the analysis of metameric variation. When, for example, a shoot with five leaves is taken from an oak tree, the leaves, ordered along the length of the shoot, differ from one another in a regular manner: The first is very variable; the second, as a rule, is of maximum length; the third and fourth are usually approximately identical; the fifth is the smallest and is very variable. In this situation the variation within the crown, measured with respect to five leaves of a single shoot, will be very great. It is expedient to take a specific leaf from the shoot, for example the third, and to collect five "third" leaves from different shoots. Furthermore, fully-developed, "normal" shoots should be collected from a specific portion of the crown, from, say, the lower third of the crown on the southern side (Krasil'nikov, 1962).

However, it is frequently virtually impossible to avoid ontogenetic differences in metameres, especially when dealing with metameric variation in animals. Two alternatives are possible here: 1) The type of dependence between trait values and successive metameres, which is known from certain theoretical considerations, is considered; 2) an empirical function is used for the systematic variations in the trait on successive metameres, which is computed as the mean for all the material. Verification of the latter alternative is especially interesting; if successful this approach may be extended to different traits of nonmetameric organs.

Usually, the variance between individuals is not used directly but in the form of a ratio to the overall variance: σ_B^2/σ^2 . This parameter in the variance-analysis scheme is called the coefficient of within-class correlation and in biological investigations is frequently designated as the coefficient of repeatability. Coefficients of repeatability have a rather large sampling error. Therefore, their estimation or comparison obligatorily involves statistical procedures (Zhivotovskii, 1979).

Until now we have been concerned with a single quantitative trait. However, the researcher is, as a rule, interested in several traits simultaneously. Very rarely are they independent, but the successive examination of the coefficients of repeatability of correlated traits is rather difficult: It is not clear whether the observed tendency for a second trait is simply determined by its correction with the first trait. This difficult problem was recently solved by L. A. Zhivotovskii (1980), who proposed the concepts of generalized variance and the generalized coefficient of within-class correlation. The author demonstrated examples of the remarkable properties of these measures for comparing similar species, to be followed by systematic between-population comparisons. Such an approach is promising on the basis of the following considerations. A quantitative trait in a population, as a rule, is determined polygenically. Let the total number of loci in a given species be N ; the first trait is determined by N_1 genes, the second by N_2 , and so on. If the genes, as often occurs, have pleiotropic effects, then many of the N_1 and N_2 genes will be common, and the study of a pair of traits by no means covers the variation with respect to the $(N_1 + N_2)$ genes. If possible, we take into account the correlations between traits, that is to say the prospect of rather completely characterizing the multitude N by increasing the number of traits from the various systems of the organism, that is to say, presenting genotype functioning as an intact system.

In spite of the fact that researchers have widely used the coefficients of repeatability for the overall ecogenetic characterization of populations, a whole series of methodological questions remains unclear. It is first necessary to estimate how stable this parameter is not only in a statistical sense but primarily in real natural populations: in repeated collections of material in a single population in different years, within a single population in taxonomically-similar species differing with respect to reproductive biology, and so on.

It is well known how our understanding of within-species variation has been expanded as a result of ecogeographic investigations of plants and animals. Undoubtedly, the use of the procedure of partitioning the variance into components will permit an important advance in the study of the population structure of species. However, as was noted above, this procedure is rather rough; it provides only the sum of the ecogenetic variation. A more advanced approach is described in the following section.

EXPRESS TEST AGAINST ECOLOGICALLY-CONTRASTING BACKGROUNDS

Insofar as in nature different individuals exist under different environmental conditions, the accurate separation of the genotypic components of variation requires as a minimum the procurement of progeny from different individuals and its cultivation under identical environmental conditions. The simplest solution here, not requiring the arrangement of crosses, consists in the performance of a family analysis: For example, seeds are collected from separate maternal plants, and entire families from different plants from the wild are sown under identical conditions; or several young are obtained from separate females trapped in the wild, and the young from all females are raised under identical conditions. Here, the quantitative trait is measured in progeny for which we know whether or not a single mother is shared. The biological features of the species studied can lead to other schemes. In several species of wild plants, for example, it is technically difficult to achieve seed germination under controlled conditions, while it is simpler to perform a cloning of the shoots, rootstocks, etc. In other species, possibly, it is technically simpler and more

precise to culture the buds, and so on. Thus, a comparison of the variation of related and unrelated individuals is the first requirement for estimating the genotypic components of variation in any population.

However, even if seeds are collected in relation to families and are sown under strictly-controlled, identical ecological conditions, this still does not mean that the differences observed between cultivated plants are conditioned only by the genotype. If, for example, two maternal plants existed under maximally-contrasting environmental conditions — "good" and "bad" — then their progeny will frequently differ from one another when cultivated under identical conditions. In annual plants such ecological aftereffects may become apparent even at the latest stages of ontogeny, as late as seed production by the offspring. These effects in perennials are apparently erased with time. Foresters, for example, know that the removal of ecological aftereffects requires 10-15 years in some woody plants. This creates difficulties not only because the period of time until the results are obtained is excessively long; it is extremely difficult to maintain the "identity" of environmental conditions for all trees for many years. Consequently, the second requirement arising in the separation of the genotypic component of variations consists in the necessity of removing the influence of the ecological aftereffect.

The third requirement is determined by general genetic concepts. Insofar as contemporary genetic thought is based on the concept of the norm of the genotypic response, it can be hypothesized (and this hypothesis requires special analysis) that the variation observable in wild populations is conditioned not only by purely genotypic or purely ecological effects but in considerable measure by the interaction between the genotype and the environment. Therefore, it is desirable that the testing of natural resources be conducted in such a manner as to estimate, however roughly, the interactions between the genotype and the environment and to compare these effects with purely genotypic effects.

The requirements formulated above are met by the following experimental scheme. From a certain number of plants, which are representative of the population, a sufficiently large number of seeds is collected from each plant with respect to family. The seeds from each plant are divided into several groups A, B, C,.... Each group from all maternal plants is cultivated under specific environmental conditions. In so doing, the variants A, B, C,... differ from one another in the fact that they are ecologically-contrasting, in some respect extreme groups. On the other hand, the sampling of extreme ecological backgrounds must provide for the removal of ecological aftereffects. In fact, it seems natural that the ecological aftereffects will be erased against various severe backgrounds, the differences between which greatly exceed the differences between maternal plants in the populations: The influences causing the aftereffects are of a different, smaller order of magnitude by comparison with the influences to which the tested plants are exposed.

On the other hand, if the progenies of the maternal plant from the population are tested against several different ecological backgrounds, identical for each maternal plant, we find ourselves in a situation for a two-factor variance analysis with a complete classification. This is a mixed model. One factor — the maternal plants 1, 2, ... — is random; the plants were taken from the population randomly and represent only a part of the population. The other factor — the ecological background A, B, C, ... — is fixed; the ecological conditions are selected arbitrarily from certain general considerations. Insofar as the number of seeds in each family is greater than unity against each background, not only the effect of the families and the effect of the ecological background can be estimated, but also the effect of the interaction between the family and background.

The analysis will be simpler and more reliable if the investigator attains uniformity in the complex, that is to say each unit in the two-factor scheme has an identical number of observations (Hicks, 1973).

If the ecological aftereffects can be removed at the earliest stages of ontogeny, then any of the quantitative traits can be recorded at this time. In this case the duration of the experiment may be limited to several weeks or months, i.e., we are dealing with an express test.

What is meant by contrasting ecological conditions? Contrasting, extreme conditions can include both conditions that are marginally tolerable for the species and those most favorable: the regimes of temperature, illumination, moisture, nutrition, and so on. Of special interest are the environmental conditions specific for a certain species or groups

of species. For example, the apparently selective growth of *Quercus pubescens* on carbonate soils is specific for the closely-related sessile-oak species *Quercus petraea* and *Q. pubescens* (Semerikov and Glotov, 1977). Therefore, the germination of acorns on a medium with an absence or an excess of CaCO_3 will be ecologically contrasting for these species. It should be borne in mind that the extreme conditions that can be selected will very often have little in common with real natural situations; however, the example cited shows how in an ideal case the selection of these conditions should be directed on the basis of the characteristics of the biology of the species.

The proposed express test characterizes the norm of the response of individual plants, while the sample as a whole characterizes the distribution of the norms of response of the population.

The use of an express test against contrasting ecological backgrounds permits demonstration of the genetic heterogeneity of populations with respect to quantitative traits in experiments lasting several weeks or months. Another question is how effective is the extrapolation of the results obtained to the traits of adult individuals. It is with respect to these traits, and among these primarily with respect to the economically valuable traits, that the estimation of the genetic potential of populations is of interest. This leads to the necessity of solving the problem of selecting in the initial stages of ontogeny those traits that would most completely reflect the traits of adult individuals, their adaptiveness. Therefore, the traits of the initial stages of ontogeny should be, as a rule, traits of adaptiveness.

A family analysis with the subsequent computation of heritability parameters, and sometimes also other populational-genetic parameters, has long been used in plant and animal breeding for estimating the genetic heterogeneity of populations with respect to quantitative traits (Rokitskii, 1974). Tests under differing ecological conditions have long been used for estimating the norm of response of a genotype (Sinskaya, 1948). The uniqueness of the express test proposed by us consists in the combining of a family analysis with a test against contrasting ecological background; in the testing of individuals under controlled, extreme conditions; in the use of a scheme of variance analysis permitting a quantitative estimation of the effects of factors and the interaction between the genotype and the environment for a specific trait; in the use of a complex of traits by the method of L. A. Zhivotovskii with the aim of characterizing the genotype as an integral system.

The express test examined may also find application in the solution of other problems. We cite two examples. In the study of polygenically-conditioned heritable human diseases the idea of extreme ecological backgrounds will result in tests against functional loads, also applicable with respect to family with, of course, the necessary corrections for sex, age, and other traits of the family members. The method of selecting plus trees in a planting, widespread in forest science, may be appreciably improved if testing for the cutting or seeds of the selected tree is conducted under extreme ecological conditions, separating the genotypic component of variation from the ecological component.

CONCLUSION

On the basis of the foregoing concerning the analysis of genetic heterogeneity in natural populations by quantitative traits, we shall examine a possible sequence in the solution of a problem involving the estimation of the genetic potential of populations within the species distribution range.

The first stage should consist in the use of published data and work experience on the characterization of the ecology and dispersal of the species; on the systematics, evolution, and natural history of the species, including information from paleontology; the biology of the species, including the system of reproduction and dispersal of individuals; and the variation in different quantitative traits. Examples of surveys of such nature are the remarkable monographs of L. F. Pravdin (1964, 1975). The result of this stage of work will be the selection for investigation of several dozens, perhaps, hundreds of populations; of methods for collecting the material within the population (sampling of individuals); methods for selecting the metameric organs of the individual; quantitative traits, considered both on metameres and in an express test; extreme ecological conditions for the express test; and the method for germinating seeds, making cuttings, etc., in the express test. The competent and careful performance of the first stage of investigations is of fundamental importance: It determines

the success of all subsequent work and is a bridge between the lasting results of the work of natural scientists of the preceding generations and the contemporary approach to the solution of the problem.

In the second stage the material is collected and the metamerism variation analyzed. On this basis, 10 to 20 populations are selected, showing the maximum ecogenetic diversity and at the same time interesting from the point of view of natural history.

In the third stage an express test is performed, making it possible to select several (up to 10) populations showing a pronounced genotypic component in the variation and interesting from the point of view of the parameters of ecogenetic interactions.

The final, fourth stage consists in the systematic investigation of the particular genetics of the selected populations, perhaps including an express test for certain F_1 combinations (within- and between-population combinations), the investigation of qualitative morphological traits, biochemical polymorphism, and so on. Thus, the study of the genetic heterogeneity of natural populations with respect to quantitative traits represents a screening procedure, permitting a more goal-directed and economical utilization of the modern laborious, expensive, and time-consuming genetic and ecological methods.

From our point of view, only a procedure of such nature, beginning from the examination of the species in the entire distribution range and then stage by stage successively using at first very rough followed by increasingly improved methods, will make it possible to obtain an objective understanding of the variation and potential of the species and, as a result, will make it possible to solve on a truly scientific basis the problems arising here in the preservation and rational utilization of natural biological resources.

LITERATURE CITED

- Ahrens, H., *Varianzanalyse*, Akademie-Verlag, Berlin (1967).
- Chetverikov, S. S., "Certain features of the evolutionary process from the point of view of contemporary genetics," *Zh. Eksp. Biol., Ser. A*, 2, No. 1, 3-54; No. 4, 237-240 (1926).
- Dobzhansky, T., *The Genetics of the Evolutionary Process*, Columbia Univ. Press, New York (1970).
- Glotov, N. V., "Genetic heterogeneity of natural plant populations with respect to quantitative traits," in: *Problems in the Ecology of the Baikal Region: Theses of Reports of a Republican Conference [in Russian]*, Irkutsk (1979), pp. 39-41.
- Glotov, N. V., "Historical outline of Soviet population genetics," in: *Genetic Research [in Russian]*, Vol. 9, Leningrad. Univ., Leningrad (1981), pp. 85-105.
- Glotov, N. V., and Semerikov, L. F., "Variation of English oak (*Quercus robur*) in Dagestan," in: *Problems in Evolutionary and Population Genetics [in Russian]*, Dagest. Filial Akad. Nauk SSR, Makhachkala (1978), pp. 78-85.
- Glotov, N. V., Semerikov, L. F., and Vereshchagin, A. V., "Natural-historical and populational investigations of the durmast oak (*Quercus petraea*) in the northwestern Caucasus," *Zh. Obshch. Biol.*, 36, No. 4, 537-554 (1975).
- Hicks, C., *Fundamental Concepts of Design Experiments*, Holt, Rinehart & Winston (1973).
- Krasil'nikov, D. I., "Variation in oaks in the Western Caucasus and its significance in taxonomy," Author's Abstract of Doctoral Dissertation, Biol. Inst. Akad. Nauk SSR, Leningrad (1962).
- Lewontin, R. C., *The Genetic Basis of Evolutionary Change*, Columbia Univ. Press (1974).
- Magomedmirzaev, M. M. (ed.), *Genetics and Evolution of Natural Plant Populations [in Russian]*, Dagest. Filial Akad. Nauk SSSR, Makhachkala (1975).
- Magomedmirzaev, M. M. (ed.), *Phenetics and Genetics of Natural Plant Populations [in Russian]*, Dagest. Filial Akad. Nauk SSSR, Makhachkala (1977).
- Magomedmirzaev, M. M. (ed.), *Problems in Evolutionary and Population Genetics [in Russian]*, Dagest. Filial Akad. Nauk SSSR, Makhachkala (1978).
- Magomedmirzaev, M. M. (ed.), *Botanical and Genetic Resources of the Flora of Dagestan [in Russian]*, Dagest. Filial Akad. Nauk SSSR, Makhachkala (1981).
- Mamaev, S. A., *Forms of Within-Species Variation in Trees [in Russian]*, Nauka, Moscow (1972).
- Mather, K., and Jinks, J. L., *Biometrical Genetics*, Chapman and Hall, London (1971).
- Nei, M., *Molecular Population Genetics and Evolution*, Holland Press, Amsterdam (1975).
- Pravdin, L. F., *Common Pine [in Russian]*, Nauka, Moscow (1964).
- Pravdin, L. F., *European Spruce and Siberian Spruce in the USSR [in Russian]*, Nauka, Moscow (1975).

- Rokitskii, P. F., Introduction to Statistical Genetics [in Russian], Vyshéishaya Shkola, Minsk (1974).
- Scheffé, H., Analysis of Variance, Wiley (1959).
- Semerikov, L. F., and Glotov, N. V., "The evolution of roburoid oaks," in: Geobotanics, Evolution, and Morphology of Plants in the Urals [in Russian], Uralsk. Nauch. Tsent. Akad. Nauk SSSR, Sverdlovsk (1977).
- Semerikov, L. F., and Glotov, N. V., "Variation in eastern oak (*Quercus macranthera*) in Dagestan," in: Plant Populations [in Russian], Leningrad. Univ., Leningrad (1979), pp. 179-189.
- Semerikov, L. F., and Glotov, N. V., "Variation in sessile oaks in Dagestan," *Ékologiya*, No. 4, 25-37 (1980).
- Sinskaya, E. N., Species Dynamics [in Russian], Sel'khozgiz, Moscow-Leningrad (1948).
- Timofeev-Resovskii, N. V., Yablokov, A. V., and Glotov, N. V., Outline of the Population Concept [in Russian], Nauka, Moscow (1973).
- Zhivotovskii, L. A., "Estimation of the coefficient of within-class correlations," *Genetika*, 15, No. 7, 1235-1242 (1979).
- Zhivotovskii, L. A., "Measures of the populational variation in a complex of quantitative traits," *Zh. Obshch. Biol.*, 41, No. 2, 177-191 (1980).