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NATIONAL POPULATION WORKSHOP:  
“ONTOGENESIS AND POPULATION”

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**IV National Population Workshop:  
“Ontogenesis and Population” (Moscow, May 18–19, 2001)**

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Papers presented at the IV National Population Workshop “Ontogenesis and Population” held in Moscow May 18–19, 2001, at the Kol'tsov Institute of Developmental Biology, Russian Academy of Sciences, are published in this issue. As follows from the title of the workshop, its goal was to unite the population and ontogenetic trends of research, which have traditionally been separate fields of study.

The estimation of developmental homeostasis in natural populations is a promising direction in population-ontogenetic investigations. This direction proves to be useful in the solution of some basic problems of population biology (paper by V.M. Zakharov *et al.* in this issue). The monitoring of populations of various plants and animals is also based on the estimation of developmental homeostasis, which proved to be effective both in nature and under the anthropogenic influence. For such estimations, morphological, cytogenetic, and physiological indices, which undergo coordinated changes, can be used (E.G. Chistyakova and N.G. Kryzheva; L.V. Sherzhukova *et al.*\*; A.K. Butoirina and E.V. Kormilitsyna; A.T. Chubinishvili; P.D. Vengerov; S.G. Dmitriev and V.M. Zakharov). Estimation of the morphological polyvariance of ontogenesis allows a better understanding of the population structure and its dynamics with respect to ecological

conditions (L.A. Zhukova and N.V. Glotov; O.V. Smirnova *et al.*\*). The use of data on genetic control of morphogenesis makes it possible to feature the pathways of morphological evolution (T.A. Ezhova and O.A. Sklyarova). Analysis of multidimensional ontogenetic channels opens new possibilities for understanding the mechanisms of appearance of the intra- and interspecific morphological diversity (M.V. Mina). Extremely important information about the history of life of an individual can be obtained through analysis of recording structures (G.A. Klevezal).

Thus, the papers published in this issue represent the current state of population-ontogenetic studies, its successes and problems, and the perspective of its use in both theoretical investigations on the field of population and evolutionary biology and in the monitoring of the environment. It is hoped that this direction will be of interest for developmental biologists and that further studies in this area will initiate new population studies.

We would like to thank most sincerely all those who helped us in preparing this issue of the journal: N.V. Glotov for his idea of organizing a series of population workshops, S.G. Vassetzky and Editorial Board of the journal for publication of the workshop materials in this issue, N.P. Zhdanova, M.S. Checheta, and reviewers for preparation of manuscripts, and W. Alton Jones Foundation, for support of the workshop and of the publication of its materials.

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NATIONAL POPULATION WORKSHOP:  
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## Ontogenesis and Population: Evaluation of Developmental Stability in Natural Populations

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**Abstract**—Ontogenetic and population studies traditionally develop independently. At the same time, the relevance of specific analysis of a specimen as ontogenesis for both correct assessment of populations and their dynamics in time and space and understanding the mechanisms underlying population processes is becoming more and more clear. Developmental stability serves as the most general description of the developing organism status. The level of developmental noise commonly evaluated by fluctuating asymmetry is the main index of developmental stability. High developmental stability is maintained on the basis of genetic coadaptation under optimal developmental conditions. Coordinated changes in developmental stability and other homeostatic indices, including genetic, physiological, biochemical, and immune indices suggest that we consider the general status of the organism by evaluating its developmental stability. Evaluation of developmental stability as a measure of environmental stress, apparently, is the most promising. In practical terms, this allows us to evaluate and monitor environmental health under both natural and various anthropogenic conditions. Outlooks of this approach as relates to studies of developmental stability are discussed.

*Key words:* ontogenesis, population, developmental stability, population variability, environmental stress, genetic coadaptation.

Individual development is the subject of ontogenetic inquiries. The subject of population studies is sets of specimen called populations. These two research trends traditionally develop independently. Specimen research evaluates the status of the organism at a given developmental stage as well as its changes in time (research of the proper ontogenesis commonly limited to embryogenesis) and space (revealing the differences between specimen). Population research evaluates the status of specimen sets at a given moment as well as its changes in time (evaluation of population dynamics) and space (revealing interpopulation and interspecific differences). Usually, it is not considered that each specimen is ontogenesis, and the evaluation of populations appears to be a section of ontogenetic tracks. When this is taken into account, the possible ontogenetic changes are considered as an interference for population assessment, and the problem is usually reduced to their obviation by considering the specimen of even age. At the same time, specific analysis of a specimen in terms of ontogenesis is essential not only for correct evaluation of the populations and their changes in time, but also for understanding the mechanisms underlying the population processes. Below, we consider the benefits of the approach based on the evaluation of the populations taking into account ontogenetic changes for population biology.

Developmental stability is among the most general descriptions of a developing organism. High develop-

mental stability is maintained on the basis of genetic coadaptation under optimal developmental conditions (Zakharov, 1987; *Developmental Stability...*, 1992; *Developmental Instability...*, 1994; *Developmental Homeostasis...*, 1997; Moller and Swaddle, 1997). The increasing number of publications on developmental stability and never-ending debates on the significance of such studies in describing the population status substantiate the assessment of applying developmental stability as a measure of environmental stress for monitoring the population status as well as analysis of failures and outlooks of this approach. This is the aim of this work.

### WHAT IS DEVELOPMENTAL STABILITY?

The evaluation of the population status is the key component of the population approach. At the population level, it is carried out using demographic indices, including abundance, age and sex composition, and reproductive success (proportion between the numbers of progeny and reproducing specimen).

In ontogenetic terms such evaluation relies on the status of specimen described by developmental stability. In this case, the problem is reduced to the evaluation of normality or perfection of developmental processes using developmental anomalies and developmental noise as the main indices. While the proper developmental anomalies—phenodeviations with considerable

changes in morphology—are extremely rare, developmental noise is an operational test for developmental stability (Mather, 1953; Zakharov, 1989). Developmental noise (Waddington, 1957)—an arbitrary spontaneous developmental variability (Astaurov, 1974) or realizable variability (Strunnikov and Vyshinskii, 1991)—is most clearly and simply evaluated from fluctuating asymmetry of the bilateral structures. The benefit of this approach relies on the known genetically determined norm—symmetry; development noise is a deviation from this norm during development. Virtually identical phenotypic effect on the left and right independently realized in the course of individual development is one of the most amazing signs of the power and accuracy of phenotypic realization of the genotype.

Two principal approaches to evaluating biological processes are possible. According to the first one, everything is strictly determined and any events that seem arbitrary to us only reflect the level of our ignorance; hence, the search for mechanisms that explain them is the actual task. According to the second one, there are allowable limits of the naturally random variability. The level of such variability reflects the system status and the impact of environmental stress. The presence of noise and fluctuating asymmetry as its most common manifestation apparently validates the second approach. Each character features the allowable limits that correspond to the essential accuracy of its realization during ontogenesis. The number of animal limbs is usually a highly canalized character, while the number of left and right bristles varies widely.

Possible distinction between noise and developmental stability seems groundless (Palmer *et al.*, 1994). There can be no noise without a developing system, and no developing system can exist without a certain level of noise. A strong system features minimal noise, while a weak one has high noise. Noise is a feature of this system that reflects developmental stability. This can be illustrated by typesetting activities. Documents cannot exist without a typesetter; they are a description of his/her work. Mistakes cannot be avoided, but their number can be minimized and depends on both the typesetter's workmanship and working conditions.

The previously recommended measure of developmental instability—phenodeviation frequency (the proper developmental disturbances or anomalies)—cannot serve as an operational index (Lerner, 1954; Rasmuson, 1960). Usually, they are quite rare due to the naturally low frequency rather than to elimination by natural selection. Experimental studies of the influence of natural factors such as temperature on various species indicate that pronounced developmental anomalies do not commonly appear within the entire thermal range suitable for development. If they do arise due to certain causes, they appear in adult specimen as well as in natural populations. Typical schemes often fail—phenodeviations do not appear after the usual environmental stress.

A normal developmental pathway of a readily recognizable organism is provided in the whole range of conditions suitable for its life. This indicates the power of canalized development. Phenodeviations resulted from unusual influences, particularly, specific chemical pollution that disturbed or simulated normal control substances—morphogenes and hormones. Certain influences induce them at once and at a large frequency. Such factors include polychlorinated biphenyls (PCB)—pesticides disturbing the activity of common biologically active compounds (endocrine disruption). For example, the frequency of significant phenodeviations such as the splitting of the parietal bone occurred in up to 30% of the offspring of American mink *Mustela vison* females intoxicated by PCB during pregnancy (Borisov *et al.*, 1997a). The phenodeviation frequency was increased by certain compounds but did not depend on the total level of the environmental stress, e.g., total anthropogenic load; their estimates did not match the fluctuating asymmetry indices. For instance, the frequency of various phenodeviants was extremely high (76%) in Moskva River roach *Rutilus rutilus*, where developmental stability was evaluated at four points (based on a five-point anomaly system) (Zakharov *et al.*, 2001). At the same time, no phenodeviants have been found in the two fish species studied at the background of very high fluctuating asymmetry in the seriously polluted region of Chapaevsk (Middle Volga), which was declared an ecological disaster zone based on our evaluations. Also, a sharply increased frequency of serious developmental anomalies has been observed in quite diverse species—invertebrates and fish—in a water basin in an area of Odessa affected by extreme eutrophication (Zakharov *et al.*, 2000a). Thus, phenodeviations indicate some specific influences cannot describe the total developmental stability. Developmental noise appears to be the only available test for developmental stability.

#### HOW CAN DEVELOPMENTAL STABILITY BE EVALUATED?

Developmental stability can be described by the level of developmental noise. In this case, the problem is reduced to considering any deviations from a known genetically determined norm. One can use various types of asymmetry, differences between homodynamic structures, and other signs of arbitrary developmental variability.

The main index is fluctuating asymmetry, which is determined to be insignificant nondirectional deviations from strict bisymmetry. This asymmetry type is usually opposed to directed asymmetry and antisymmetry with strictly inherited commonly significant differences between sides (Ludwig, 1932; Van Valen, 1962; Neville, 1976). Despite the attractiveness of the proposed evolutionary relationship between fluctuating asymmetry, antisymmetry, and directed asymmetry (Mather, 1953), it has no genetic and morphogenetic

grounds (Zakharov, 1987). In the presence of the morphogenetic gradient to the left and right of the body axis and its absence from left to right, the fluctuating asymmetry is a manifestation of arbitrary developmental variability—a certain difference in independent phenotypic realization of the character on the left and right. According to a vivid phrase coined by Levontin (1978), only one unsuccessful attempt of selection in *Drosophila* is known—the conversion of fluctuating asymmetry to the directed one. Increasing stresses makes identical independent realization of a character on the left and right improbable. This significantly increases the frequency of specimen with a more pronounced character on the left or right as compared to the symmetric variants. There are no grounds to propose the emergence of a specific biological phenomenon (antisymmetry). All the more, such bimodal distribution of the differences between sides (signed) in a studied sample of specimen inevitably appears with increasing genetic or environmental stress and disappears without it (McKenzie and Clarke, 1988; Leary and Allendorf, 1989).

Evaluation of developmental stability by each character consists in accounting for asymmetry or differences in character values on the left and right. Generally, any morphological characters suitable for precise measuring can be used. In the case of meristic character, the asymmetry value in each specimen is determined by the difference in the number of structures on the left and right, while, in the case of a plastic character, it is calculated as the difference in the measurements on the left and right relative to total measurements on the both sides. Application of such a relative value evens out the asymmetry value dependence on the character value. The population estimate is expressed as the mean arithmetic.

A set of morphological characters should be used to evaluate total developmental stability. Integral indices of developmental stability should be used to analyze a complex of characters. In the case of meristic characters when the number of certain structures is counted, the mean frequency of asymmetric manifestation per character (FA) is the simplest and most reliable measure:

$$FA = (\sum X_i)/n,$$

where  $X_i$  is the number of asymmetric characters in each specimen divided by the number of used characters and  $n$  is the number of specimens in the sample.

In the case of plastic characters related to metering, the average relative difference between the sides per character (RD) can be recommended as the integral index. It is calculated from the same formula, where  $X_i$  is the total relative difference between the sides (difference between measurements on the left and right divided by total measurements on the both sides) of all characters divided by the number of used characters.

These indices give us an integral description of the fluctuating asymmetry by all considered characters. They depend considerably less on the value of the particular difference between the sides and individual deviation variants as compared to other indices. Such an approach obviates many complications related to the variance and arithmetic mean of the absolute difference between the sides or correlation between the sides widely used previously (also by us; Zakharov, 1987). The statistical significance of the difference between the samples of the integral indices of developmental stability is determined from *t* Student's test.

Calculation of the indices per character allows comparison of the data obtained for a different number of characters. Since characters can have a different asymmetry, the evaluations obtained for particular characters can differ. However, when we use a set of characters (e.g., ten) not specifically selected by the level of variability and asymmetry, such integral evaluations appear comparable. For instance, the values of developmental stability obtained from cranium characters proved to be similar for both close and distant species such as voles, seals, and aurochs (Zakharov *et al.*, 2000b).

A certain difference can be recorded when comparing samples, and its statistical significance can be evaluated. Evaluation of the degree of the revealed deviations and their place in the total range of the index can be inconvenient. Such evaluation is particularly important for comparing various territories and species. Processing the data for various natural populations can include development of a scale system reflecting deviation from the norm. Below are the basic principles of its building. The range of index values corresponding to the conventional background norm is taken as one point (conventional norm), while that corresponding to the critical condition is taken as five points. The whole range between these threshold levels is ranked in increasing order of the index. In practice, the first point corresponds to stable development under optimal conditions in laboratory experiments and natural populations not subjected to pronounced stress. Five points correspond to the results obtained under serious stress with a pronounced unfavorable effect on viability. Since the data on a number of independent indices are summed, we indeed have an integral evaluation. Such a system is a scale evaluation of changes in the organism status by developmental stability. Such scale systems are now available for integral indices of developmental stability for some plant, fish, amphibian, and mammalian species (Zakharov *et al.*, 2000b). Some examples of such scale evaluations are presented below.

#### DETERMINATION OF DEVELOPMENTAL STABILITY: GENOTYPE AND ENVIRONMENT

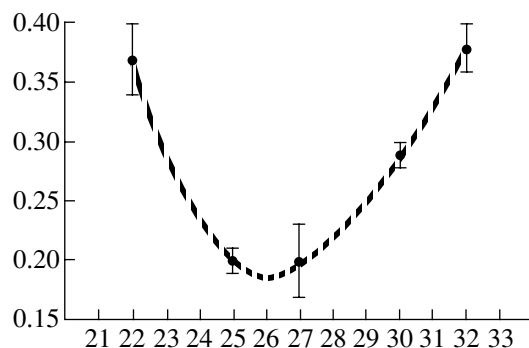
From the beginning, the developmental stability test was mainly based on fluctuating asymmetry and was carried out separately for genetic and environmental factors. Consideration of fluctuating asymmetry as a

developmental noise raised the problem of its possible dependence on any factors (Waddington, 1957) and, if so, the problem of the key factor (genotypic or environmental) of the noise level.

*Genotype*

The first publications on the genetic determination of fluctuating asymmetry raised the problem of its possible dependence on the general properties of genotype rather than particular locus even for an individual character (the number of sternopleural bristles in drosophila was given close attention) (Mather, 1953; Thoday, 1958). Total homozygosity and later genetic coadaptation were proposed as such properties (Zakharov, 1987, 1989; Clarke, 1993; Developmental Homeostasis..., 1997). Although asymmetry increased in some cases of high homozygosity, the relationship between developmental stability and genetic properties can be presented generally as its independence from genetic coadaptation. The following data confirm this: (1) in some cases increased homozygosity has no notable effect on fluctuating asymmetry; (2) fluctuating asymmetry of various characters clearly depends on some loci but does not depend on others. This suggests that the presence or absence of such a relationship depends on locus selection; (3) sometimes certain changes in chromosomes or even single loci can affect developmental stability manifested as the increased asymmetry of various characters; (4) numerous data on hybridization of various forms demonstrated that developmental stability depends on the gene combination in the genotype rather than on the total heterozygosity level. This can be presented in the following form: (1) hybridization of close forms with relatively high developmental stability does not affect this property (as observed after crossing closely related laboratory strains, natural populations, and even close, particularly, twin species); (2) hybridization of close forms with low developmental stability increases developmental stability via a cancelled inbred depression due to increased homozygosity (which is commonly observed after crossing closely related highly homozygous laboratory strains); (3) hybridization of remote forms with considerable genetic differences adapted to various conditions decreases developmental stability (as observed after crossing remote strains as well as intra- and interspecific forms). At the same time, even the reverse trend can be observed—decreasing developmental stability with increasing heterozygosity (Graham and Felley, 1985).

Note that high homozygosity *per se* does not disturb developmental stability and the general status of the organism. This is due to an increased sensitivity to changed developmental conditions and the transition of unfavorable alleles to the homozygous state. Specific experiments on drosophila and silkworm (Astaurov, 1974; Strunnikov and Strunnikova, 2000) demonstrated constant viability indices under optimal developmental conditions (temperature, feeding, population density, etc.)



**Fig. 1.** Relationship between developmental stability and the incubation temperature of sand lizard *Lacerta agilis* eggs; abscissa: incubation temperature, °C; ordinate: integral index of developmental stability (mean frequency of asymmetric manifestation per character evaluated for 13 meristic characters of follidosis).

when “bad” genes were specifically removed. Hence, this research trend suggests that developmental stability evaluated by fluctuating asymmetry of individual characters depends on genetic coadaptation.

*Environment*

Evaluation of the influence of environmental factors indicates disturbed developmental stability in conditions of environmental stress. Figure 1 exemplifies experimental evaluation of the relationship between developmental stability and incubation temperature for the sand lizard *Lacerta agilis* (Zakharov, 1987, 1989, 1993). Fluctuating asymmetry is clearly minimal only under certain conditions; it increases both after increasing and decreasing temperatures, thus indicating decreased developmental stability. Similar data were obtained in studies on the temperature effect on certain insect and fish species (Thoday, 1953; Leary *et al.*, 1992; Clarke and McKenzie, 1992). Decreased developmental stability was observed during mouse and rat prenatal and early postnatal development (Siegel *et al.*, 1992) under the influence of decreased and increased temperatures as well as in various species in conditions of increased density, social stress (Valetsky *et al.*, 1997), and under the influence of various chemical pollutants (Valentine and Soule, 1971; Graham *et al.*, 1993; Borisov *et al.*, 1997a).

Generally, evaluation of the natural factor (in this case, temperature) indicates that the dependence of developmental instability indices has the form of a curve with the minimum corresponding to the optimum. In the case of unfavorable factors with a zero optimal value (e.g., chemical pollution), the relationship has only one branch of this curve. This corresponds to decreased developmental stability with increasing influence of the unfavorable factor. Note that an increase in fluctuating asymmetry in conditions of disturbed developmental stability can be traced by both

the integral index and individual characters related to quite diverse morphological structures (Zakharov, 1987, 1989). This is also indicated by experiments on the effect of PCB on the mink organism (Borisov *et al.*, 1997a). Disturbed developmental stability is observed by three independent character groups: the number of openings in the cranium, the torus palatinus number, and planum nasolabiale dermatoglyph. Limitation of a further increase in fluctuating asymmetry indices reported in some cases after environmental stress exceeded a certain critical level (Clarke and McKenzie, 1992) is not that significant, since the consequences of such a strong influence can be revealed by other approaches.

The dependence of developmental stability on both genetic and environmental factors can be illustrated by two experiments on different animal groups: the Chinese silkworm *Bombix mori* and the rat *Rattus norvegicus*.

The relationship between developmental stability and the incubation temperature was tested for three silkworm strains considerably differing in their heterozygosity (Zakharov and Shchepotkin, 1995). A certain level of total heterozygosity has been obtained and fixed in these strains by meiotic and ameiotic parthenogenesis. The relationship between developmental stability and temperature proved to be uniform for all strains, and the curves are parallel. Developmental stability is minimal for highly homozygous strains and maximal for low homozygous strains at all thermal modes.

Another experiment on laboratory rat strains demonstrated the relationship between developmental stability and certain genetic factors as well as social stress (Borisov *et al.*, 1997b). The homo- and heterozygosity of an individual locus has no effect on the developmental stability of an inbred strain, and further inbreeding decreases it. Hybridization of closely related strains increases the developmental stability, while hybridization of remote strains decreases it. Social stress also decreases developmental stability in the initial strain.

Hence, developmental stability depends on both genetic and environmental stress. The notions of genetic and environmental stress largely converge. A changed environment makes the previous genetic coadaptation inefficient, and, conversely, disturbed coadaptation makes previously optimal conditions nonoptimal for a given genotype. The response to any unfavorable influences by both genotype and environment appear the same—the organism status changes which are monitored by a disturbed developmental stability. Only the nature of the inducing factors can distinguish environmental or genetic stress. Developmental stability evaluates the organism status depending on both the system power (genetic coadaptation) and environmental conditions (environmental stress). Developmental stability serves as an ontogenetic description of the organism

status or a morphological measure of genetic coadaptation and the effect of environmental stress.

## DEVELOPMENTAL STABILITY IN NATURAL POPULATIONS

Studies on developmental stability in natural populations are promising for revealing the changes in developing organism in conditions of genetic or environmental stress.

### *Genetic Stress*

The possible evaluation of genetic changes rely on revealing disturbed genetic coadaptation. Such cases are rarely revealed by screening natural populations. In the majority of cases, genetic coadaptation is maintained in natural populations. It can be disturbed by hybridization in zones of secondary intergrading of genetically distinct forms that have adapted to considerably different conditions (Zakharov 1987, 1993). Most such zones are known. In this case, the focus is testing whether such hybridization disturbs genetic coadaptation rather than revealing the zones. Single positive (Clarke, 1993) and multiple negative cases have been reported (Jackson, 1973; Felley, 1980; Lamb *et al.*, 1990; Chubinshvili, 1997). One should not expect genetic coadaptation after all types of hybridization allowed in natural populations for a significant number of generations.

A high homozygosity level can also affect developmental stability. Such cases are also rare. Most known examples are somehow related to anthropogenic influences. These include a sharply decreased species abundance, semiartificial breeding, and populations isolated from the bulk of the species. This can be exemplified by decreased developmental stability in aurochs in the Belaya Vezha population, which was restored from a small group of founders. Decreased developmental stability in this population is not yet accompanied by a decrease in other adaptation indices, while a more serious disturbance of developmental stability is accompanied by greatly changed viability in another population from Plessk (due to an even higher inbreeding level) (Baranov *et al.*, 1997). Standard genetic tests indeed demonstrated high homozygosity in this case (Hartl and Pucek, 1994). In other cases, a pronounced decrease in homozygosity was not accompanied by decreased developmental stability (Fouler and Whitlock, 1994; Hartl *et al.*, 1995). This raised anew the question of whether fluctuating asymmetry can be considered an index of developmental stability and the status of developing organism as a whole. Actually, increased homozygosity had no effect on the developing organism status in this case. This indicates yet again that the organism status is not affected by any changes in heterozygosity. Evaluation of developmental stability is merely the most simple and adequate response to the question whether changed heterozygosity level is

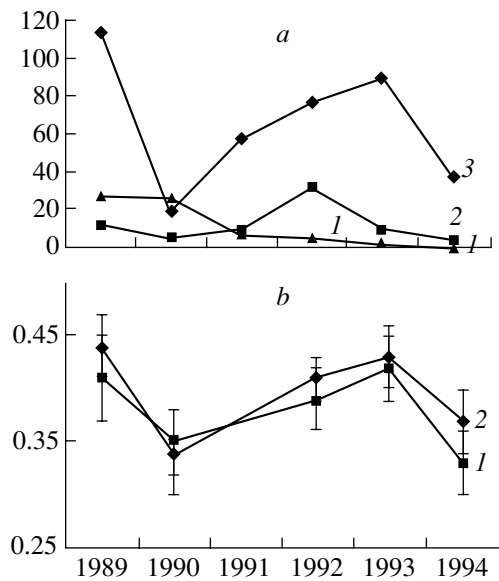
accompanied by a changed organism status in each particular case.

Another interesting situation for such studies is possibly disturbed developmental stability due to changed genetic coadaptation at a certain stage of microevolution—during genetic changes on the way of adaptation to new conditions. This can be exemplified by a well-known disturbed coadaptation when survival in the presence of an insecticide is allowed (Clarke, 1993). Evaluation of developmental stability in this case demonstrated if general disturbance of genetic coadaptation possibly affecting the general status of developing organism (detectable by various phenotypic characters) is observed in this case.

*Environmental Stress*

Investigation of developmental stability as an indicator of environmental stress offers much greater possibilities for studying natural populations.

**Population dynamics.** Population dynamics remain one of the key problems in population biology. Within the considered frames, the following main problems can be mentioned: how population status is changed during the population cycles; what is the relationship between these changes and population density; what is the role of biotic and abiotic factors; and what is the role of the density in changes in population size. The studies were carried out on a common shrew *Sorex araneus* (Zakharov *et al.*, 1991). The feedback between developmental stability and abundance have been revealed in the cyclic population of Central Siberia with assumed abundance autoregulation. This means that high developmental stability is specific for a relatively low population density, while disturbed developmental stability can be observed for top abundance due to overpopulation, which subsequently decreases the population. Later similar data were obtained for five other true species with synchronous population dynamics (Zakharov *et al.*, 1997a; Dmitriev *et al.*, 1997). This assumption has been confirmed by the data obtained for two vole species. Changes in developmental stability of these species proved to be independent of their abundance but correlated with the abundance of shrew species defining the small mammal community in the studied habitat (Fig. 2). Indications of positive association of developmental stability and abundance have been found for a cyclic population in southern Finland. In this case, the reverse effect is observed: developmental stability decreases with decreasing abundance resulting from unfavorable environmental conditions (e.g., climatic effect). Increased abundance below the overpopulation threshold (which has stress effect) is accompanied by high developmental stability under favorable conditions. Such a situation is also specific for invertebrates. Their abundance increases under favorable environmental conditions (Zakharov, 1987). The data obtained indicate promising application of this approach for revealing the mechanisms underlying



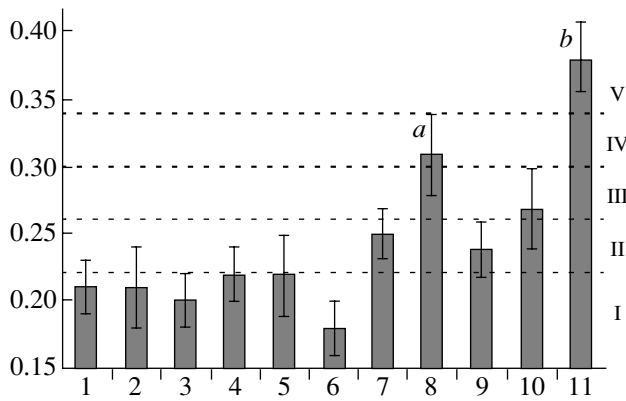
**Fig. 2.** Time-related changes of abundance (a) and developmental stability (b) of various small mammalian species from Central Siberia (Turikhansk Region); 1, large-toothed red-backed vole *Clethrionomys rufocanus*; 2, northern red-backed vole *Clethrionomys rutilus*; 3, eight shrew species; abscissa: years; ordinate: (a) population density (a number of caught specimen per 100 cylinders per day); (b), integral index of developmental stability (mean frequency of asymmetric manifestation per character evaluated for 10 cranium characters).

population dynamics: positive association of developmental stability and density indicates the important role of environmental factors, while the negative one supports autoregulation cycling and the stress effect of overpopulation.

Time-related changes of developmental stability under the influence of the anthropogenic factor can be exemplified by investigation of Baltic seals. Analysis of museum collections (developmental stability was evaluated by fluctuating asymmetry of cranium characteristics) demonstrated the disturbance during the most serious pollution of the Baltic Sea from the 1960s to the 1970s, as compared to the preceding and consecutive periods (Zakharov *et al.*, 1997b). DDT and PCB pollution was considered the main cause of this change and was confirmed by the influence of experimental PCB exposure on developmental stability in mink. Progeny of minks exposed to PCB indicates decreased developmental stability (Borisov *et al.*, 1997b). The possibility of evaluating developmental stability using solely morphological characteristics suggests possible monitoring of population status even by investigation of museum collections.

**Interpopulation differences.** One of the most interesting tasks of interpopulation variability studies is the evaluation of the range periphery, particularly, the ecological rather than geographical periphery. Decreased developmental stability indicates a degraded





**Fig. 3.** Changes in developmental stability towards northern ecological periphery of the sand lizard *Lacerta agilis* range; (a), region of chemical pollution; (b), ecological periphery of the range; abscissa: number of a population sample from south (Rostov Region) to north (Leningrad Region); I–V, point scale (here and below); ordinate: integral index of developmental stability (mean frequency of asymmetric manifestation per character evaluated for 13 meristic characters of follidosis).

status of the organism at the ecological periphery of the range (Soule and Baker, 1968; Kat, 1982; Zakharov, 1987). For instance, investigation of the sand lizard *Lacerta agilis* demonstrated disturbed developmental stability increasing towards the northern ecological periphery of the range (Fig. 3). Note that the conditions of ecological periphery are now widespread due to the anthropogenic influence. The same example demonstrates that the northward gradient of developmental stability is sharply disturbed by anthropogenic stress factors. The conditions of the range ecological periphery can appear in different parts of the range also due to natural reasons; this is most clearly seen in plants. For instance, developmental stability of the weeping birch *Betula pendula* is considerably lower under shady conditions as compared with light conditions (Zakharov and Krysanov, 1996).

This substantiates population studies aimed at revealing the role of geographic isolation and environmental conditions in the formation of intraspecific phenotypic variability. Developmental stability is an important operational test in defining optimal habitats for the studied species. This is particularly important for widespread species common and abundant in diverse geoclimatic zones and with an unknown history of origin. Such investigations were conducted for hemipteran *Graphosoma lineatum* widespread in various zones. Comparison of various populations demonstrates a decreasing integral index of developmental instability with decreasing humidity and increasing insulation, which can be traced for both the whole range of the species and within each zone: forest, steppe forest, and steppe (Fig. 4). Gaps in the presented histogram correspond to the absence of steppe biotopes in the forest zone and forest biotopes in the steppe zone.

It is important that many populations, including spatially distant ones, feature similar levels of developmental stability. At the same time, its deviation can be observed after considerable environmental changes within minor territories. This suggests that, despite certain differences, many populations of a species have a similar optimum of developmental stability, which allows us to recognize populations under nonoptimal conditions. Each species is adapted to specific conditions, and the populations where “something started” can be revealed on the periphery of the range; disturbed developmental stability is the price for existing under new conditions.

**Various species.** Comparison of sympatric populations of various species in order to reveal their response to the same conditions is most interesting. Such data have been obtained for insects and reptiles (Zakharov, 1987). Different responses indicate that the same conditions appear optimal for one species but nonoptimal for another. The existence of such differences indicates that “something has happened,” and, due to some adaptive changes, the population can safely exist under new conditions.

#### DEVELOPMENTAL STABILITY AND GENERAL PHENOTYPIC VARIABILITY

Rough assessment of the nature of the observed phenotypic variability is extremely important in population studies. Such an assessment is hard to obtain for natural populations. Commonly genotypic variability is assumed to be the main cause of phenotypic variability. This is only partially true, and sometimes it is not true at all. A significant portion of general genotypic variability (up to 50% and more) in natural populations can result from developmental noise (Zakharov, 1987). It can largely define the dynamics of phenotypic variability observed in natural populations. The above-mentioned data on population dynamics of the common shrew in various regions exemplifies this. The level of noise ontogenetic variability changes in parallel to general phenotypic variability in acyclic population of southern Finland, while in the Central Siberian cyclic population the noise variability sharply increases during stress and largely determines phenotypic variability for the top abundance (Zakharov *et al.*, 1997c).

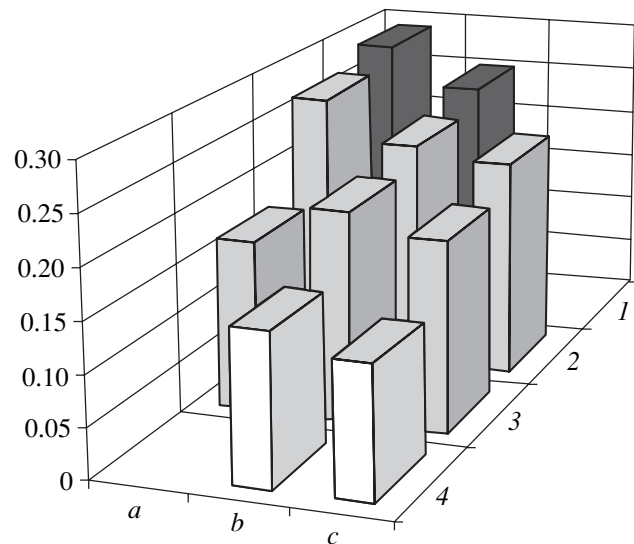
#### DEVELOPMENTAL STABILITY AND EVOLUTION

Two aspects of developmental homeostasis should be considered for assessment of the mechanisms underlying microevolutional transformations: both stability and canalization or plasticity of development as the capacity to develop a similar phenotypic effect under different environmental conditions (Mather, 1953; Zakharov, 1987). This allows experimental assessment of the natural events with an account of specific envi-

ronmental factors. The simplest approach for such assessment is to incubate the offspring of parents from different parts of the range at different temperatures (Zakharov, 1993). For instance, the relationship between the wing length and temperature is the same in two fruit flies *Drosophila subobscura* populations (Norway and Italy); the curves go parallel with the shift towards modifications similar for both populations. Significant interpopulation differences for each thermal mode indicate genetic determination of a new reaction norm. Similar data were obtained for *Lacerta* lizards. The relationship between the number of femoral pores and the temperature was also similar in all studied forms; the curves are parallel but shifted towards the modifications: from the widespread sand lizard subspecies *Lacerta agilis exigua* to the southern subspecies *L. a. boemica* and further to the southern species five-streaked lizard *L. strigata*. These data support the mechanism of appearance of modern interpopulation and interspecific differences on the basis of genetic determination of a new reaction norm appearing on the basis of modification response. Evaluation of developmental stability demonstrated that the response of one form to increased temperature can be reproduced in another form by decreased temperature and at the background of high developmental stability. The above-mentioned example of the comparison of two species in nature points to the above microevolutional process that made optimal previously nonoptimal conditions.

Hence, the evolutionary mechanism can be initiated by ontogenetic changes rather than mutations. According to classical population genetics, nothing happens in this case. The main stages of evolutionary transformation can be presented as follows.

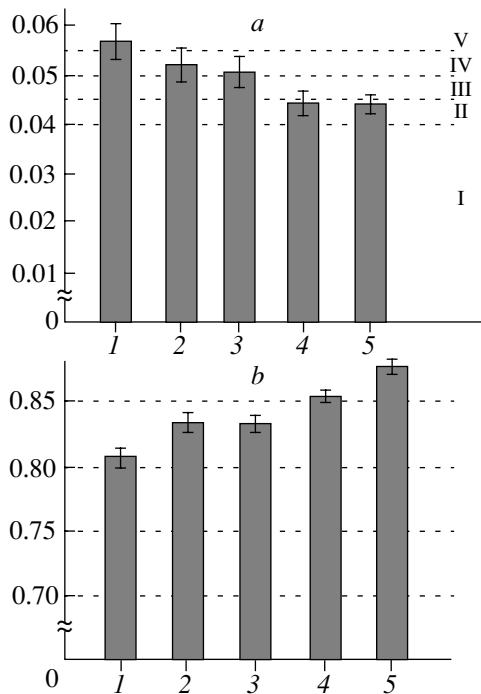
The initial situation features high developmental stability of the population with high genetic coadaptation under optimal conditions (first stage). Stress influence of new conditions decreases developmental stability even in the first generation with the same genotype due to the direct response of the organism to environmental influences (second stage). Long-term existence of the population under these conditions (for numerous generations) usually induces adaptive genotypic changes, allowing both survival and adaptation to the new conditions; as a result, the stress influence of the environment is considerably decreased. However, homeostasis is not yet improved, apparently, largely due to the genetic stress. This can be explained by disturbance of the previous genetic coadaptation by the genotypic changes providing for survival under new conditions (third stage). The subsequent genotypic transformations can lead to a new genetic coadaptation and reconstitution of high developmental homeostasis, which indicates conversion of nonoptimal condition to the optimal ones (fourth stage).



**Fig. 4.** Developmental stability of hemipteran *Graphosoma lineatum* from various habitats; abscissa: (a), forest; (b), steppe forest; (c), steppe; 1–4, various habitats arranged by increasing insolation and decreasing humidity (recognized on the basis of geobotanical description of the habitats); ordinate: integral index of developmental stability (mean frequency of asymmetric manifestation per character evaluated for eight pigmentation characters).

The whole process including the above four stages is a microevolutional transformation on the way to adaptation to new conditions. This changes the optimum; i.e., initially nonoptimal conditions become optimal. Clearly, this process is not always completed and can be stabilized at any stage. Such cases can be described as the ecological periphery of the range. Decreased homeostasis in these populations is the price for survival under new conditions at the survival boundary of the species.

Unfortunately, the process can hardly be traced from beginning to end, due primarily to the considerable time intervals. However, the reality of the described process is indicated by comparison of various populations being at different stages. Disturbed developmental stability at the ecological periphery of the range, as compared to the populations under normal conditions reported many times (Soule, 1967; Soule and Baker, 1968; Zakharov, 1987), corresponds to the initial stages (second and third) of the above process. The difference between developmental stability of sympatric populations of closely related species most likely indicate the transition from disturbed to restored developmental stability in the course of the adaptation process (from third to fourth stage). Disturbed developmental stability at the ecological periphery of the range for a widespread species and high stability for a specialized species indicate different responses to the same conditions. The sympatry zone is an ecological periphery that disturbs developmental stability for one species and an optimal



**Fig. 5.** Indices of developmental homeostasis in the samples of weeping birch *Betula pendula* from various sites (region of Chapaevsk); forest belts: 1, town territory (between the fertilizer plant and the “Polymer” factory); 2, near the fertilizer plant; 3, near the “Polymer” factory; 4, 3 km away from town (conventional control); 5, 10 km away from the town (conventional control); ordinate: (a), integral index of developmental stability (mean frequency of asymmetric manifestation per character evaluated for five leaf measurements); (b), efficiency of photosynthetic processes (alternating to maximum fluorescence ratio).

habitat with high developmental stability for another one.

#### THE MEANING OF DEVELOPMENTAL STABILITY

Ample data on possible changes of developmental stability evaluated by fluctuating asymmetry of individual characters naturally raises the question of their significance: do they describe the specific development of an individual character or morphogenesis as a whole, to what extent do they describe general stages of the organism, and can they be used to evaluate the status of a species population, community, and ecosystem as a whole.

##### *Relationship between Developmental Stability Estimated by Different Characters*

Information on general developmental stability can be obtained even with an individual character; however, a set of characters provides for a more reliable evaluation. A specific feature of fluctuating asymmetry is the absence of a relationship between differences of the

sides even in highly correlated characteristics in individuals such as leaf measurements in plants. Another illustrative example is the number of gill-rakers on four gill arches in fish. With the correlation of these characters close to the unity, the relationship between their asymmetry does not reliably differ from zero (Zakharov *et al.*, 2000b).

At the same time, in terms of population tests, the asymmetry of different characteristics usually appears highly correlated. Their coordinated changes are a reliable indication of the differences in general developmental stability in the studied group of samples. Vice versa, in the absence of such differences, deviations of asymmetry by individual characters are stochastic and, naturally, are not coordinated (Zakharov, 1987). This can be exemplified by the data on the effect of PCB on minks (Borisov *et al.*, 1997a). In this case, coordinated changes in the asymmetry value have been revealed both within each of the studied character groups and for different groups (coordination of these changes is confirmed by the Friedman test,  $p < 0.05$ ).

##### *Relationship between Developmental Stability and Other Indices of Developmental Homeostasis*

Coordination between changes in developmental stability and other indices of the organism status describing various aspects of developmental homeostasis is extremely important for understanding the significance of these changes.

Below are several examples. Disturbed developmental stability of the Chinese silkworm after decreased heterozygosity and stress influence of increased or decreased temperatures (Zakharov and Shchepotkin, 1995) is accompanied by similar changes in another physiological index of homeostasis—efficiency of energy metabolism or total oxygen consumption required for passing certain developmental stages (Aleksieva and Gubanov, 1993). Despite the increased duration of development and decreased metabolic rate at decreased temperatures, the minimum total oxygen consumption is only allowed at certain thermal conditions that can be described as optimal. The optimum zones for energy metabolism efficiency and developmental stability proved to coincide. Hence, in this case, we observed the relationship between asymmetry of wing measurements and total oxygen consumption. Another example is the relationship between increasing asymmetry of cranium characters and increasing frequency of chromosome aberrations in somatic cells under the influence of increased density in populations of small mammals (Dmitriev *et al.*, 1997). Experiments on social stress in rats demonstrated that increasing asymmetry of cranium characteristics is accompanied by changes in the immune status (Valetsky *et al.*, 1997; Pronin *et al.*, 1997).

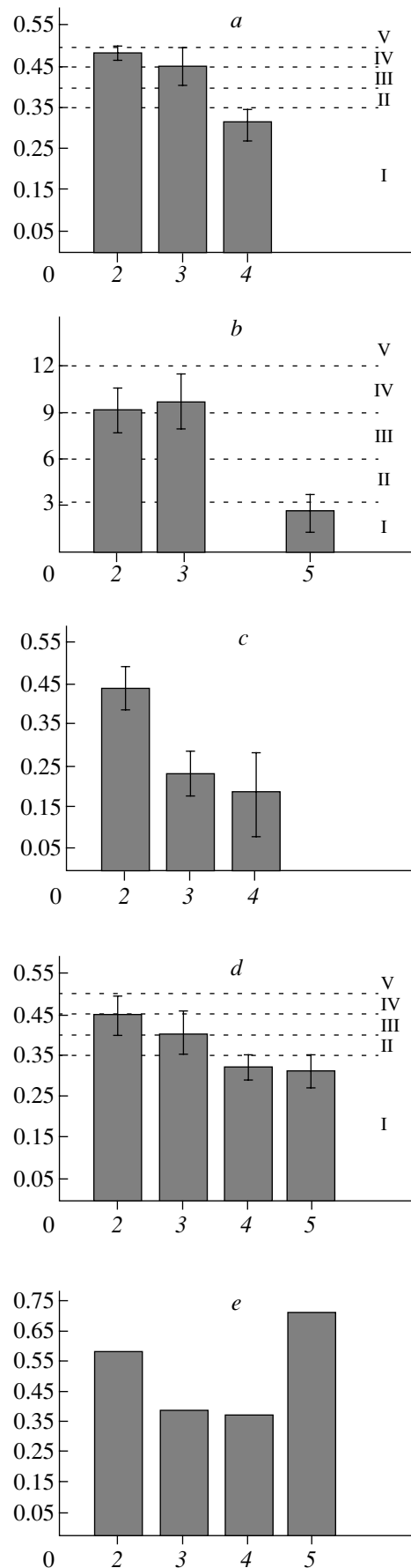
Multiple examples of such coordinated changes of quite diverse homeostatic indices have been obtained

during investigation of anthropogenic factors (Zakharov *et al.*, 2000a): (1) asymmetry of leaf measurements increased with a decreasing rate of photosynthesis in plants (Fig. 5); (2) asymmetry of cranium characteristics increased with the frequency of chromosomal aberrations in somatic cells, indices of immune status, and superoxide dismutase activity (a marker of oxidative stress and growth processes in mammals) (Fig. 6).

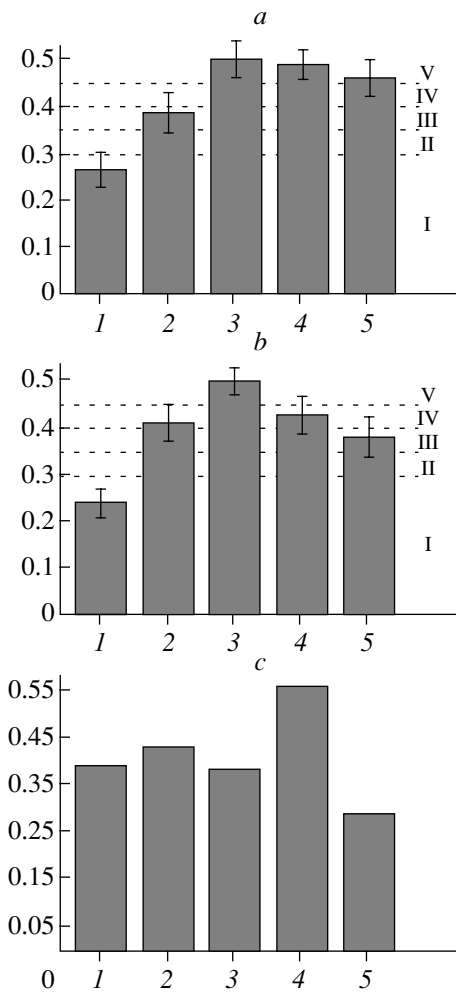
In general, coordinated changes of various organism indices can be revealed by five main approaches: morphological, biochemical, genetic, immune, and physiological (Biotest..., 1993; Zakharov and Krysanov, 1996; Zakharov *et al.*, 2000a). Coordinated changes in all of these indices are always observed after disturbed homeostasis. This coordination is a reliable indication of changed developmental homeostasis and the general status of the organism after genetic or environmental stress. In all studied cases, the changes of various indices of the organism activity were accompanied by changes in developmental stability. Importantly, it follows that the revealed changes in developmental stability indeed reflect the changes in the general status of the organism.

*Relationship between Estimates of Developmental Stability and Adaptation Indices*

Multiple examples of the relationship between developmental stability and commonly used indices of the organism adaptation indicate its possible application for rough assessment of adaptation (Clarke, 1995; Moller, 1997). This seems promising for describing the status or health of the organism rather than for accounting the progeny and contribution to the next generation (which is quite complicated for natural population studies). The benefits of such an approach include integrity and sensitivity of the test; in addition, it is operational and widely applicable. The significance of the approach can be exemplified by experimental evaluation of the PCB influence on American mink *Mustela vison* (Borisov *et al.*, 1997a). The control developmental stability in dead-born mink was considerably lower (high



**Fig. 6.** Indices of developmental homeostasis and species variability of small mammals in sites 1–5 (see Fig. 5); ordinate: (a), integral index of developmental stability in the samples of common red-backed vole *Clethrionomys glareolus* (mean frequency of asymmetric manifestation per character evaluated for ten cranium characters); (b), cytogenetic homeostasis in the samples of common red-backed vole *Clethrionomys glareolus* (frequency of aberrant cells, %); (c), immune status in the samples of common red-backed vole *Clethrionomys glareolus* (the mean deviation of certain immune indices); (d), integral index of developmental stability in the samples of Caucasian wood mouse *Apodemus uralensis* (mean frequency of asymmetric manifestation per character evaluated for 10 cranium characters); (e) species diversity (Shannon's index of species diversity for eight small mammalian species).



**Fig. 7.** Developmental stability in the samples of bream *Abramis brama* (a) and roach *Rutilus rutilus* (b) (mean frequency of asymmetric manifestation per character evaluated for seven meristic characters) and species diversity of fish (c) (Shannon's index of species diversity for 11 species) in various sites: 1, river region upstream from town; 2, lake within the town's territory; 3, river region adjacent to the fertilizer plant; 4, river region of waste discharge; 5, river region downstream from the waste discharge site.

asymmetry) than compared with live-born ones, while after experimental intoxication both had a similarly high level of developmental disturbances. Hence, disturbed developmental stability takes place when the organism status is degraded due to various factors but is not the cause of its death. This allows us to use this index as a fine marker of organism status.

Application of various approaches to the evaluation of developmental homeostasis demonstrated their coordinated changes with developmental stability (Zakharov and Krysanov, 1996; Zakharov *et al.*, 2000a). Hence, measuring developmental stability describes both the morphogenetic processes and the general status of the organism. In addition, these evaluations proved to be correlated with the proper popula-

tion index—reproductive success. Such a correlation has been observed during the population cycle of the common shrew *Sorex araneus* (Zakharov *et al.*, 1991). In addition, an unexpected synchronous decrease in developmental stability observed during the subsequent investigation in five *Sorex* species was confirmed by decreased indices of reproductive success (Zakharov *et al.*, 1997b). Generally, this confirms the significance of such evaluation for describing the status of population and its higher sensitivity as compared to other approaches.

#### *Relationship between Developmental Stability Indices in Various Species*

The evaluated stress effect of the environment applies only to a given species, while changed developmental stability can be observed only in this species. At the same time, if the effect of a given factor applies to a group of species, similar response can be observed in various species. This can be exemplified by a similar response in various small mammalian species on overpopulation as indicated by synchronous population dynamics in these species (Zakharov *et al.*, 1997b). It is even more pronounced in the case of a stress factor affecting virtually all species of the considered ecosystem. This is mostly pronounced for anthropogenic (chemical or radiation) pollution. In this case, we can see a similar disturbance in developmental stability in quite diverse as well as in close species of the same group, but also in different groups. Presently, we know many such facts (Zakharov *et al.*, 2000a), e.g., in the chemically polluted Middle Volga region. An even value of developmental stability disturbance is observed for plants and mammals in terrestrial ecosystems as well as for fish and amphibians in aquatic ecosystem (Figs. 5–8). Coordinated changes in organism status indices for various species confirm the reliability of the obtained results and allow us to develop a test for the health of the environment by the status of inhabiting species on the basis of developmental stability analysis.

#### DEVELOPMENTAL STABILITY AND EVALUATION OF ENVIRONMENTAL HEALTH

This approach proved to be promising for practical application and for background monitoring (under natural conditions) and evaluation of the consequences of the anthropogenic influence. The principal advantage of this ontogenetic approach is its capacity to reveal the changes in the organism status for various pollution types when either biodiversity (at the level of communities) or population indices usually indicate no change (Zakharov and Krysanov, 1996; Zakharov, 2000a).

This approach allows us to evaluate the health of the environment and is understood as the evaluation of its

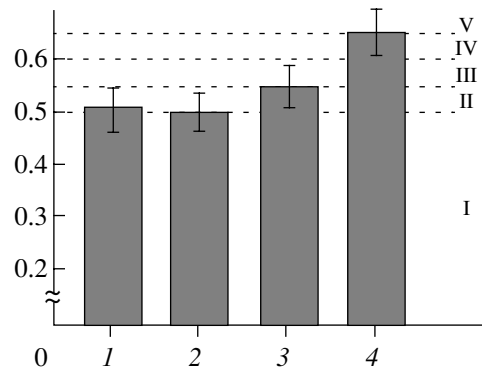
favorability for life forms, including humans. Why health? Because the evaluation of environmental quality is carried out by the health of the inhabiting life forms. In short, the proposed approach consists of the evaluation of an organism's status by developmental homeostasis as the most general property of a living organism's activity. In scientific terms, this approach can be defined as the evaluation of natural populations status by the health of the constituting organisms, while in practical terms it can be defined as the evaluation of the health of the environment.

This can be exemplified by the above-mentioned data obtained by various approaches for various species in the Middle Volga region (Figs. 5–8). Generally, such evaluations were carried out in various regions with various anthropogenic influences including chemical, radiation, industrial, and agricultural pollution as well as complex anthropogenic influences. Evaluation of deviation from the norm is the principal point. The five-point scale has been developed for the main approaches of the proposed methodology. As a result, we can evaluate deviation of the organism status from a given index within the frames of each approach (morphological, genetic, and immune). Comparison of the points obtained for various approaches in various species demonstrated that the scale is universal for most species, and the data obtained with various approaches commonly give a similar picture. This allows us to propose scale evaluation of a living organism status as a whole (Zakharov *et al.*, 2000a).

As a result, the revealed picture appears similar despite certain differences between the data obtained by various indices and species. Instead of the usual mosaic of biological responses by different methods and for different species, the main indices of the proposed approach show a correlated response. This correlation of responses is one of the basic principles of the proposed methodology for evaluating the health of the environment and always occurs when two conditions are satisfied: all used indices describe the organism status by developmental homeostasis and real change of such characteristic takes place in the considered group of samples. Coordinated changes of various ontogenetic noise indices for different morphological characters indicate a changed general developmental stability. Coordinated changes for different approaches for the evaluation of developmental homeostasis indicate changed general status of the organism. Coordinated responses of various species indicate a changed environmental health.

The proposed methodology for evaluating the status (health) of living systems by developmental homeostasis is not just one of the methods; rather, it is a completely new approach to the problem of the evaluation and monitoring of the environment.

The evaluation of the health of the environment allows us to reveal consequences of an influence even



**Fig. 8.** Integral index of developmental stability (mean frequency of asymmetric manifestation per character evaluated for 13 meristic characters) in the samples of marsh frog *Rana ridibunda* from various sites (see Fig. 7).

when they are undetectable by other methods. Serious changes in organism status are often observed at the background of invariable biodiversity. The general conclusion of such ecological studies is constant or increased level of biodiversity and abundance of individual species in the zones of chemical or radiation pollution as compared to the neighboring territories. For instance, the level of biodiversity of small mammals and fish proved to be higher compared to conventional control territory at the background of extremely serious disturbances in developmental stability (Figs. 6 and 7). Several causes of such an effect can be mentioned. Chiefly, the species living there sense no such influence. In addition, they cannot be replaced by other species, since these conditions are optimal for no other species. The main factor of species extinction is physical change of the habitat, and such human activity is very limited in the pollution zones. Factories are often built in extremely rich habitats (e.g., flood planes). Another factor for maintaining reach biodiversity is eutrophication common in such places, which is also attractive for many species.

The approach based on the assessment of marker species is not universal either. For instance, widely used marker species—frogs and crayfish—were very abundant in highly polluted region of the town of Chapaevsk. Importantly, the evaluation of environmental health can reveal the deplorable state of numerous representatives of various species exercising their ecosystem function at the background of unaltered landscape.

The similarity of the results obtained with different approaches suggests rough estimating of the organism status using several or even one single approach. The morphological approach based on the evaluation of developmental stability can be recommended as the most promising for wide application due to its high sensitivity, relative simplicity, and coordination with other approaches in evaluating the organism status.

DESCRIPTION OF THE APPROACH BASED  
ON EVALUATION OF DEVELOPMENTAL  
STABILITY: COMPLICATIONS AND OUTLOOKS  
FOR FURTHER DEVELOPMENT

The increasing number of publications on developmental stability, on the one hand, and never-ending debates on the significance of such studies and the applicability of fluctuating asymmetry as an indicator of developmental stability and developmental stability as an indicator of organism status, on the other hand, point to the specific consideration of this method.

The power and promising use of any approach clearly depend on the successful solution of methodical problems and valid causality of the considered properties.

The first and, probably, main reason for obtaining controversial data on developmental stability (chiefly related to evaluation of fluctuating asymmetry) is the ambiguity of obtaining primary data, i.e., measurements and miscalculations of morphological characters. While the theoretical foundation of the work and, particularly, interpretation of the obtained data as well as the methods of their statistical processing are considered in detail, the methods of primary data obtaining are commonly not discussed. Actually, the method is quite simple and assumes only accurate counting or measurement of the characters on two sides of the body. At the same time, researchers know considerable complications of realization of a problem with simple definition. Accurately defining the limits for measurements or critical size of the structures for calculation can be quite complex. Their overcoming assumes both a strict description of the method for accounting for morphological characters and experience in such work. In the case of other methods, e.g., accounting for the frequency of chromosomal aberrations in somatic cells, one does not dare to obtain a lot of data and publish the results (particularly, those conflicting the generally accepted notions) without training in an expert laboratory in this field, while the seeming simplicity of the evaluation of fluctuating asymmetry provokes gathering a lot of data personally or with the help of assistants without due regard for the reliability of the data obtained. Intercalibration [TAH1] of the methods for obtaining the primary data in order to obtain comparable results through joint or parallel treatment of the same material by representatives of different research groups involved in the fluctuating asymmetry investigation by a certain system of characters in similar species could largely obviate the theoretical discussion on possible genetic and morphogenetic mechanisms that underlie developmental stability required to explain controversial data.

The importance of this problem is understood from the experience in our laboratory. Many specialists ask us for methodical assistance. The general conclusion of this work is that obtaining reliable data applicable to compare with the data obtained by other research

groups is only possible with practical skill. Practical recommendations do not suffice in this case. We can exemplify this by our recent attempt to popularize the method for evaluation of developmental stability in ten conservations. Various subjects of inquiry—from plants to mammals—were used there. Verification demonstrated that reliable data were obtained only in three conservations where most researchers had experience with such work; quite controversial data were obtained in the other ones. Data collection improved only after cooperation with our specialists. Without such special methodical training, several publications could be prepared stating that no expected results were obtained and raising once more the question of the applicability of this approach. Essentially, the approach should be methodically justified with an emphasis on obtaining the primary data rather than statistical processing, which can be quite simple and is realized without much trouble in practice. Further expansion of the approach should rely on both development of experimental techniques and establishing centers for specialist training.

Methodical justification of the approach naturally raises the question of its application scope and possible interpretation of the obtained data. Any approach requires such theoretical justification. The theoretical justification seems evident for a methodically complex investigation, but it is particularly important for a simpler one. Such methods include evaluation of developmental stability by morphological variability.

First, one should note how the approach is justified. We believe that the approach based on evaluation of developmental stability is now justified theoretically and confirmed by practical investigation (Zakharov, 1987, 2000a; Developmental Homeostasis..., 1998; Moller, 1997). Further accumulation of examples is hardly sensible. The general conclusion is that developmental stability is an ontogenetic index of the organism status and changes after genetic or environmental stress. In other words, the absence of the desired result—differences in developmental stability—should be considered a sign of the absence of the studied genetic or environmental variations on the organism status determined from developmental stability. Attempts to question the application of fluctuating asymmetry in order to describe developmental stability and, consequently, the application of developmental stability to describe the organism status resemble presently attempts to question their application in describing karyotype or biochemical processes in the absence of differences by cytogenetic or biochemical tests. Thus, evaluation of developmental stability should not be used to confirm an already established situation; it should rather have the research nature similar to any other approach.

Next, one should clearly define the range of questions that can be answered by evaluating developmental stability. Examples of changed developmental stability after hybridization and pollution do not mean that

developmental stability can be used to reveal zones of hybridization or to evaluate pollution. This can be more reliably determined by other approaches. Evaluation of developmental stability can only provide unique information on the changes in the developing organism, possibly accompanied by certain genetic or environmental changes. This is the significance of developmental stability investigations. Certain genetic and environmental changes with consequences clearly revealed by other approaches (for instance, investigation of phenodeviations) can have no effect on general developmental stability, while the consequences of genetic or environmental stress for the general status can be determined from developmental stability. The simplicity and unambiguity of the assessment also applicable to natural populations is the unique property of the approach. The reliable solution of the above-mentioned methodical problems provides a positive outlook on the evaluation of developmental stability for solving these problems.

Note that developmental noise is the main index of developmental stability. It is hardly sensible to trace the adaptive significance of each determined difference in a morphological character between body sides or any change in the noise level. The observed morphological differences can hardly decrease significantly the organism viability; rather, they are important only as markers of changes in the organism general status. The enthusiasm of such attempts and even positive results are amazing. It may be appropriate to quote the famous Russian geneticist Chetverikov (1968): "Taxonomy knows thousands of examples when species differ by neutral (in biological science) rather than adaptive characters; attempts to find adaptive significance for all of them is an evenly inefficient and ungracious work when you do not know what is more amazing—the endless wit of the authors or their belief in the endless naivety of the readers." At the same time, significant changes in the noise level, which can be overlooked with other methods, are a positive indication of initial changes in the organism's general status that should later surely affect other commonly used adaptation indices.

All this defines the outlooks of developmental stability studies. As with other approaches, this one has a strictly defined range of problems that can be efficiently solved by it. Further extension of the already available multiple examples is hardly sensible. Undoubtedly, the desired effect will be obtained for correct settlement of the problem and methodical accuracy of its solution. Adequate application of the approach is the main point. We believe that this is the most efficient measure to stop agitation of the approach and to provide its wide practical application. Developmental stability is a measure of genetic and environmental stress. In these terms, the approach seems promising for a number of problems in both experimental conditions and natural populations. Considering that genetic stress is quite rare, particularly in natural populations, this index mostly serves as a measure of environmental stress. Evaluation of the

health of the environment by ontogenetic description of the composing species seems the most promising method for the approach application.

## CONCLUSION

Quite recently, we have seen evidence of the very important introduction of genetic views in population studies. Popular warnings that the organism was not a bag of beans (genes) were largely translated into reality. At the same time, the insistent demand to put on "genetic glasses" considerably distorted the vision of many population researchers—changes in the genotype were seen behind all phenotypic changes. The importance of "ontogenetic glasses" is becoming more and more clear; population researchers experience degrading vision without them. Understanding that the path from genotype to phenotype depends on developmental stability, and canalization allows us to understand that not all genotype changes affect the phenotype and not all phenotype changes result from genotypic ones.

Apparently, further development of this approach can reactivate population studies clearly declined now on the basis of traditional population genetics. Generally, development of the approach of analyzing natural populations while taking into account ontogenetic changes is very important for the further development of population studies from phenomenology to the mechanisms and causality of the observed differences, transition from isolating populations and species and population genetic models of pollution and speciation to evaluation of population status, its dynamics, and the mechanisms of evolutionary transformations.

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## Possible Application of the Indices of Developmental Stability and Photosynthetic Activity for Studying the States of Natural Plant Populations using the Weeping Birch as an Example

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**Abstract**—We have generalized the materials of population-phenogenetic studies of the weeping birch in different environmental conditions using developmental stability analysis and photosynthetic activity measurements.

*Key words:* plants, population, developmental stability, fluctuating asymmetry, photosynthetic reactions.

Developmental stability is one of the most general characteristics of development, which specifies the ability for minimization of random developmental disturbances (Mather, 1953). It was shown that the highest level of developmental stability was observed under conditions optimal for the existence of living beings (Zakharov, 1982). Developmental stability is reduced under conditions of environmental or genotypic stress (Zakharov, 1987).

The estimate of developmental stability was proposed as a general and unspecific measure of the state of organisms (Biotest, 1993). The authors proposed that the estimates obtained when using the developmental stability indices should characterize the state of the organism as a whole.

The aim of this work was to check the above suggestion. In order to solve this task, it is necessary to receive parallel estimates using different approaches. We analyzed developmental stability in the natural population of the weeping birch *Betula pendula* Roth., and the results were then compared to the data obtained using the induction of chlorophyll fast fluorescence. This method is widely used to study the state of plants based on their photosynthetic activity (Karapetyan and Bukhov, 1986; Lichtentaler and Rinderle, 1988; Veselovskii and Veselova, 1990; Levenko *et al.*, 1990), and we believe it is the most convenient solution for the above task.

### MATERIALS AND METHODS

The weeping birch *Betula pendula* Roth. widespread in Eurasia, was chosen for these studies. Leaves were taken for analysis from shortened shoots in the lower part of the crown of generative birch trees located on the margins of forest strips and on forest glades. Five

(or ten in samples from Chapaevsk) were collected from each of nine to ten trees in each sample. The morphological structure of the leaves was fully formed by collection. The same leaves were used to analyze developmental stability and to estimate the primary photosynthetic processes.

In order to assess the influence of natural stress conditions on developmental stability and photosynthetic activity in natural weeping birch populations, we compared samples of leaves from birch trees located in Oka State Reserve in a glade in conditions of maximum illumination and under a forest canopy (1). Sample 1 included 50 leaves; sample 2 included 45 leaves. The samples were collected in September 1995.

The birch is characterized by high respiration intensity (Tsel'niker, 1978). The level of energy expenditures does not allow long-term existence under a forest canopy with small biomass increments, while, under nursery conditions, the birch is characterized by high photosynthetic intensity and, correspondingly, high productivity (Evstigneev, 1991, 1994). Thus, the birch has a narrow range of productive capabilities, i.e., low shade tolerance and high light requirements, hence, its existence in forest communities is only possible in the most illuminated habitats (Chistyakova and Evstigneev, 1994). Therefore, we propose that the shady conditions will be stressful for the birch.

It is also essential to evaluate the influence of the anthropogenic effect as one of the most widespread stress factors in determining the dependence of developmental stability and photosynthetic activity on various environmental changes. In an attempt to solve this issue, we studied the effects of chemical contamination of the environment and nonionizing irradiation on developmental stability in natural populations of weeping birch.

In order to estimate the chemical and environmental contamination, we analyzed serial samples from Chapaevsk, Saratov District, which were collected in May–June 1994. Chapaevsk is the former center of chemical and other weapons production (Fedorov, 1994). At present, several chemical factories in the town are operational. Leaf samples were collected for analysis at five sites located different distances from chemical contamination sources in Chapaevsk and adjacent territories. Site 1 was chosen for estimating the simultaneous effect of two factories and the total anthropogenic stress of the town. Sites 2 and 3, located in the direct vicinity of the factories, were chosen in order to characterize the effects of the main sources of chemical contamination. Sites 4 and 5 (recreation zones located 2 and 10 km outside of town, respectively) were chosen as conditional controls. A total of 69, 74, 100, 99, and 99 leaves were analyzed in sites 1–5, respectively.

An experiment was conducted in 1995 at the Zhukovo Experimental Station in the Kaluga District to study the effects of nonionizing radiation. We studied samples of birch leaves from two neighboring regions in the birch forest margin. One region was subject to the effect of electromagnetic fields (1) and the other was used as the control (2). Fifty leaves were collected for analysis from each region.

The experimental region was subjected to the electromagnetic effects of radio waves as described below. Irradiation was performed in a pulse regime (duration 0.6  $\mu$ s, frequency 1 kHz, power 120 kW) daily from April 28 to June 10 and then from July 1 to July 10. The irradiation lasted 4 h per day (from 6 am to 8 am and from 5 pm to 7 pm). This regime allowed us to obtain at the moment of irradiation an intensity equal to 30  $\mu$ W/m<sup>2</sup> in the center of experimental region and 15–20  $\mu$ W/cm<sup>2</sup> at the edges.

The fluctuating asymmetry value was used as an index of developmental stability, which was defined as a result of the incapacity of plants to develop along a strictly determined path and expressed as small nondirected deviations from the ideal symmetric state (Zakharov, 1987). The differences in the character expression observed in the case of a fluctuating asymmetry cannot be fully reduced either to genotypic or environmental differences, since the character development proceeds at identical environmental conditions and on the basis of the same genotype. Thus, the expression of fluctuating asymmetry can be considered a consequence of random disturbances in the course of development and the fluctuating asymmetry itself, an expression of random developmental variability and a measure of developmental stability (Astaurov, 1927, 1930).

We estimated the fluctuating asymmetry of bilateral characteristics of the leaf morphology, which allowed us to carry out our investigation without harming the plant, and used the characteristics that were fully

formed at the time the study was conducted. This permitted precise and unambiguous estimates (Kryazheva *et al.*, 1996).

Since the leaf sizes varied greatly, the fluctuating asymmetry for each character was estimated according to the ratio of the differences between the left (*L*) and right (*r*) measurements and the sum of measurements ( $(L-R)/(L+R)$ ).

The integral indicator of the fluctuating asymmetry was the mean relative difference between the sides of each character (the arithmetic mean of the ratio of the difference and the sum of the left and right measurements was considered as one of the characteristics).

The differences in the indices used for the different samples were estimated using Student's *t*-test (Sokal and Rohlf, 1981).

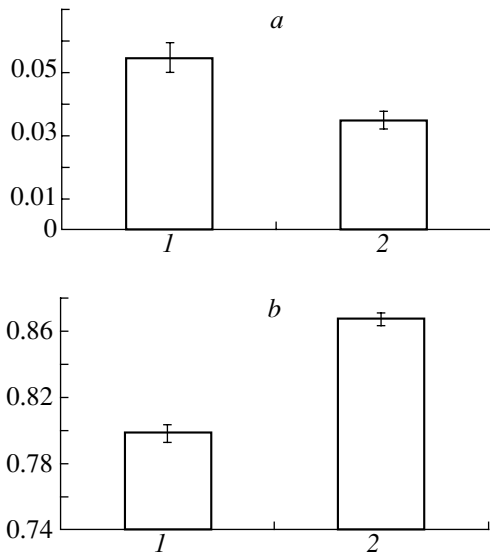
For evaluation of the photosynthetic system state, we used the method of induction of the fast fluorescence of the chlorophyll. The fluorescent response was recorded from the upper leaf surface, and fluorescence was measured using a compact computerized fluorimeter CFF-1000 (Morgan, USA). Fluorescence was invoked using the focused light of a 35 W halogen lamp, and an inbuilt microprocessor automatically recorded the parameters of the induction fluorescence curve. Before measurement, the leaves were adapted in darkness for 3 min, and the intensity of photosynthetic reactions was estimated according to the relative yield of alternating fluorescence ( $F_v/F_m$ ).

The differences in the indices used for the different samples were estimated using Student's *t*-test (Sokal and Rohlf, 1981).

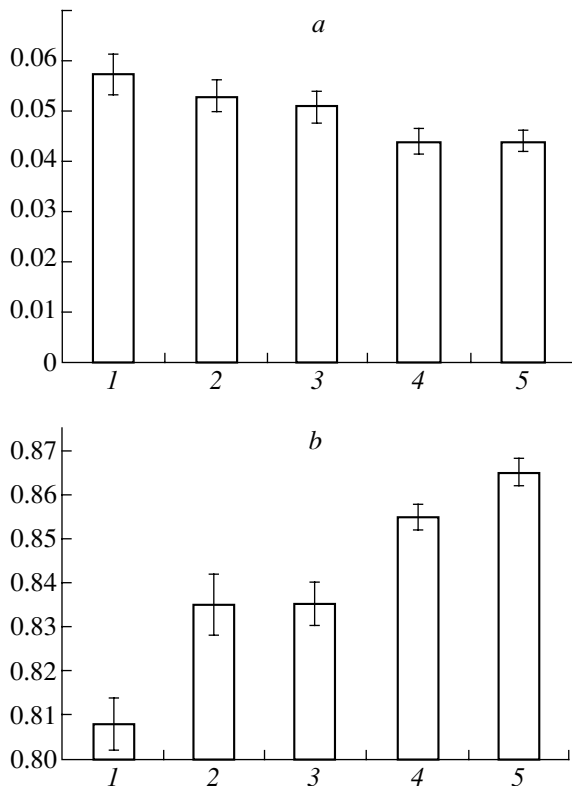
## RESULTS AND DISCUSSION

*Effects of natural unfavorable actors on developmental stability.* The value of the integral index of developmental stability amounts to  $0.055 \pm 0.0047$  and  $0.035 \pm 0.0029$  in samples 1 and 2, respectively ( $p < 0.01$ ). A higher level of fluctuating asymmetry in the sample of birch trees growing under the forest canopy (Fig. 1a) suggests reduced developmental stability under conditions of significant shade accompanied by decreased photosynthetic activity (Fig. 1b). The value of  $F_v/F_m$  in the sample from the shaded region (1) amounted to  $0.798 \pm 0.0051$  and was significantly lower ( $p < 0.001$ ) than in the trees growing in the glade in conditions of maximum illumination (2) ( $0.867 \pm 0.0027$ ), thus suggesting decreased photosynthetic activity in the former. The indices of developmental stability and photosynthetic activity that we used gave similar results and allow us to evaluate shade conditions as stress.

*Effects of chemical contamination on developmental stability.* The mean relative difference between the sides per characteristic in the samples from Chapaevsk was  $0.057 \pm 0.0040$ ,  $0.053 \pm 0.0033$ ,  $0.051 \pm 0.0031$ ,  $0.044 \pm 0.0026$ , and  $0.044 \pm 0.0022$  in sites 1–5, respectively. The samples from control sites 4 and 5 differed



**Fig. 1.** Fluctuating asymmetry (mean relative differences between the side per character) (a) and index of efficiency of the photosynthetic processes (Fv/Fm) in samples of weeping birch *Betula pendula* Roth. Under conditions of weak (1) and strong illumination (2).



**Fig. 2.** Fluctuating asymmetry (mean relative differences between the side per character) (a) and index of efficiency of the photosynthetic processes (Fv/Fm), (b) in samples (1–5) of weeping birch *Betula pendula* Roth. At different levels of anthropogenic stress (see text).

significantly from those at sites 1 ( $p < 0.001$ ) and 2 ( $p < 0.05$ ) (Fig. 2a).

The samples in question can be divided in three groups according to this index. In our case, the highest level of developmental stability disturbances was observed at the most contaminated site, which was subject to the simultaneous influence of two chemical plants and the total anthropogenic pressure of the town (1). It was somewhat lower in the zones of influence of each of these plants and the lowest at the control sites (4 and 5).

The use of the photosynthetic activity index confirmed the results obtained when analyzing developmental stability. The Fv/Fm value increased from site 1 (center of the town) to site 5 (considered as a conditional control) (Fig. 2b) and was similar at sites 1–5:  $0.808 \pm 0.0057$ ,  $0.835 \pm 0.0067$ ,  $0.835 \pm 0.0055$ ,  $0.855 \pm 0.0032$ , and  $0.865 \pm 0.0035$ , respectively. The sample from site 1 differed significantly from those at sites 2 and 3 ( $p < 0.01$ ) and from those at control sites 4 and 5 ( $p < 0.001$ ). The samples in question can also be divided into three groups according to the photosynthetic activity index. The lowest index was observed at the most contaminated site 1, while it was somewhat higher in the zone of influence of each of the factories. The samples collected at sites 4 and 5 (conditional control) had similarly high values. The morphological and physiological indices we used proved to be highly correlated ( $r = 0.9095$ ,  $p < 0.01$ ). The levels of developmental stability and photosynthetic activity decreased under conditions of chemical contamination.

*Effects of nonionizing irradiation on developmental stability.* The mean relative difference between the sides per characteristic was higher in the experimental region (1) than in the control (2):  $0.053 \pm 0.0038$  versus  $0.042 \pm 0.0034$  ( $p < 0.05$ ) (Fig. 3a). These results suggest that the developmental stability was disturbed by nonionizing irradiation.

The fluorescence curves in the control and experimental regions were analyzed twice: on July 3, 2 h after termination of the last irradiation (the first series of samples), and on July 7, four days after irradiation (second series of samples).

In the first series of samples, significant differences were found between the experimental and control regions ( $p < 0.01$ ). The value of the index that we used was higher in the control than in the experiment:  $0.839 \pm 0.0043$  versus  $0.821 \pm 0.0041$ . In the second series, the differences between the experimental and control regions were statistically insignificant. The Fv/Fm value was  $0.832 \pm 0.0034$  and  $0.834 \pm 0.0035$ , respectively (Fig. 3b). The differences in the fluorescence curves measured directly after irradiation suggest a decreased efficiency in the photosystem II electrontransport chain under the influence of electromagnetic fields. The absence of significant differences in the second series (four days after irradiation) suggests the possible recovery of the damaged photosystem II

reaction centers and of the electrotransport chain (its efficiency did not differ from that in the control). Thus, we can speak of reversible inhibition of photosynthetic activity under the influence of the electromagnetic field.

On the whole, the data we obtained also suggest a parallel decrease in developmental stability and photosynthetic activity under the influence of nonionizing irradiation.

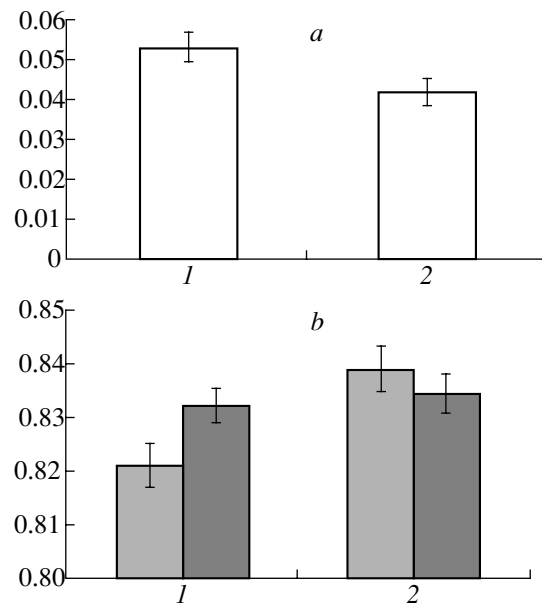
As a result of population studies on developmental stability in the weeping birch, it was found that the highest level was inherent in the plant population not subjected to evident stress. Developmental stability decreased with the increased influence of various unfavorable actors (significant shading and chemical and radiation contamination of the environment).

Since plants of the same population may grow under significantly different environmental conditions, which is due to the attached mode of life, the population may be heterogenous by developmental stability: for example, the birch trees growing in shade have a lower developmental stability than the well illuminated trees. Comparison of the indices of developmental stability and photosynthetic activity in the natural weeping birch populations has shown that the indices used provide, on the whole, similar information on the state of plants. The increased level of morphological disturbances, i.e., increased level of fluctuating asymmetry, is accompanied by a decreased efficiency in the primary photosynthetic reactions (Fv/Fm) under various unfavorable influences. Both indices suggest an inhibited state of the organism induced by an increasing stress effect.

The parallel changes we described in different indices, such as the allometry of leaf measurements and intensity of photosynthetic processes, suggest that developmental stability is a general characteristic, whose changes are expressed as deviations in diverse functional parameters. Methodically, the results obtained suggest a possible application of morphogenetic indices for evaluation of the general state of the organism.

However, the data obtained with the use of two approaches (estimation of developmental stability and photosynthetic activity) may in some cases not coincide. Such discrepancies are, above all, due to the different nature of these indices. The index of photosynthetic activity characterizes to a greater extent plant productivity, rather than its general state. At the same time, no unambiguous relationship was found between productivity and developmental stability in a special study. The highly productive tobacco varieties had the mean, rather than high, level of developmental stability (Sakai and Shimamoto, 1965).

Second, the index of photosynthetic activity (Fv/Fm) is more dynamic, reflects the state of the plant photosynthetic system at a given moment of time, and can change rather rapidly under the influence of environmental factors (Karapetyan and Bukhov, 1986). The



**Fig. 3.** Fluctuating asymmetry (mean relative differences between the side per character) (a) and efficiency index of the photosynthetic processes (Fv/Fm) (b) in samples of weeping birch *Betula pendula* Roth. from the region subjected to electromagnetic irradiation (1) and from the control region (2) 2 (□) and 4 h (■) after irradiation.

recovery of reduced photosynthetic activity after elimination of the disturbing influence is also possible, as we observed in experiments with nonionizing irradiation. The morphological index of developmental stability, fluctuating asymmetry, was formed for a long time and depends to a lesser extent on random variations in environmental factors.

Thus, a more detailed comparison suggests that it is more advantageous to use the developmental stability index for estimation of the plant state. The estimation of developmental stability can be recommended as an informative index for monitoring of natural plant populations occurring in both natural conditions and under anthropogenic stress.

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## Cytogenetic and Anatomic Features of the Weeping Birch in the 30-km Zone Surrounding the Novovoronezh Nuclear Power Plant

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**Abstract**—We studied the development of the vegetative and generative spheres in the weeping birch from the 30-km zone surrounding the Novovoronezh nuclear power plant. Being the most sensitive ontogenetic stages, the mitotic and meiotic processes are capable of responding to the effects of various factors, including unfavorable environmental factors. Studies were carried out from 1998 to 2000 in Novovoronezh under conditions of potential hazard for normal development (a possible joint effect of chronic ionizing irradiation at low doses from the Novovoronezh nuclear power plant and elevated concentrations of some chemical substances as side products of the plant activity) and in the ecologically pure territory of the Biological Station Venevitinovo of the Voronezh State University. Significant deviations from the normal course of mitotic and meiotic processes have been recorded in the experimental variant and specific types of disturbances have been described. A suggestion has been put forward concerning some mechanisms underlying the weeping birch adaptation to unfavorable environmental conditions. Specifically, it was shown that the trees studied were of a mixoploid nature. No such studies have ever been carried out in the region of this nuclear power plant, one of the oldest in Russia.

*Key words:* weeping birch, Novovoronezh nuclear power plant, mitosis, meiosis during microsporogenesis, mitotic index, nucleolar activity, mitosis pathologies, mixoploidy, stomata.

Under conditions of potential ecological hazard of nuclear power plants, cytogenetic monitoring has acquired a special significance, which allows the indirect estimation of the environment state (Biotest..., 1993; Bol'shakov *et al.*, 1995; Mezherin, 1996; Bykh and Malkina, 1996). The use of trees as test-objects gives, as a rule, good results when diagnosing technogenic contamination, since they are convenient for biomonitoring and, as perennial plants, are subject to the chronic effect of pollutants (Biotest..., 1993; Dmitrieva, 1996). Artificial plant populations are most susceptible to the influence of unfavorable anthropogenic factors, since the natural populations are more stable.

Different developmental stages of plants differ in their sensitivity to stress factors. The meiotic and mitotic stages of tissue differentiation are the most sensitive. Studies of these stages are of great interest, since their results allow us to estimate the state of the environment according to the condition of the generative sphere of trees and of the vegetative sphere of their descendants and reveal some mechanisms of plant adaptation at the cellular level under extreme conditions. Hence, we studied the course of these processes in the birch tree under conditions of complex environmental contamination by radioactive and chemical substances. Studies were carried out in the territory of Novovoronezh located in the direct vicinity of Novovoronezh nuclear power plant (NV NPP) (4 m to the

east) from 1998–2000. No such studies have previously been carried out in the region of this nuclear power plant, which is one of the oldest in Russia.

### MATERIALS AND METHODS

The choice of the territory of Novovoronezh as potentially hazardous in the ecological respect was determined based on the following considerations. First, there is information about the radioactive pollution of soils of the NV NPP region by radionuclides  $^{134}\text{Cs}$  and  $^{137}\text{Cs}$  of the Chernobyl origin, as well as by the radionuclides whose presence is due to the activity of the plant itself: periodically occurring  $^{57}\text{Mn}$ ,  $^{60}\text{Co}$ ,  $^{58}\text{Co}$ , and  $^{59}\text{Fe}$  (their activity is insignificant) and constantly occurring  $^{40}\text{K}$ ,  $^{226}\text{Ra}$ , and  $^{232}\text{Th}$  (Ernestova *et al.*, 1993; Egorov *et al.*, 1994; Bezopasnost'..., 1994; Negrobov, 2000). We assume that the cumulative effect of chronic irradiation at low doses could have arisen during the 37 years of the NV NPP activity.

Second, the territory of Novovoronezh is also markedly contaminated by chemical substances: the atmosphere pollution with dust is significant and exceeds 64 units of the total pollution index (TPI), the main contribution coming from zinc, tin, lead, and phosphorus compounds (92% of TPI), and the level of nitric oxides is elevated in some regions of the town. The total pollution of the soil cover corresponds to the mean level



(14.8 TPI units), while the regions with abnormally high pollution (more than 32) tend to occur in the western part of the town, adjoining the NV NPP cooler pond (we laid down an experimental plot in this part of Novovoronezh). The main pollutants are strontium, lead, zinc, chrome, and lithium (Kozlov, 1993). This allows us to suggest combined contamination of the territory in question with radioactive and chemical substances.

The ecologically pure territory of the Biological Station Venevitino, where natural populations of the weeping birch occur, was chosen as the control. The experimental plot laid down in Novovoronezh is located far from the public road, which practically excludes the possibility of partial explanation of the results we obtained by the influence of exhaust. Well developed trees with the normal phenotype, five in each variant, were chosen for investigations. Weeping birch seeds were collected for studying the mitotic processes in the end of August–middle of September for two years (1998 and 1999) from the same layer. Freshly collected seeds were grown in Petri dishes with the addition of a small amount of sodium permanganate at the room temperature. The materials were fixed in a mixture of ethanol and glacial acetic acid (3 : 1) at 9 am (peak of mitotic activity). The roots were stained by acetohematoxylin or 35–40 min. Forty squash preparations were made of germlings and examined: 20 from germlings of 1998 seeds and the rest from germlings of 1999 seeds. A total of 19 033 cells were analyzed. Forty preparations were also made from germlings of the seeds collected in the ecologically pure territory (20 for 1998 and the rest for 1999). A total of 17 532 cells were analyzed. The following indices were used for characterization of mitotic processes: mitotic and nucleolar activity, frequency and spectrum of mitotic pathologies, and micronuclear test. They could serve as criteria for cytogenetic monitoring (Cherepanova and Aleksakhin, 1971; Biotest..., 1993; Arkhipchuk, 1995).

Male catkins were collected for studying meiotic processes during microsporogenesis in 2000. Meiosis starts in the weeping birch, as a rule, in the last ten-day period of July (Yurevich and Gel'tman, 1963; Shiman'yuk, 1964; Ermakov, 1986), but, in 2000, this process started much earlier due to the weather: early and warm spring. The male catkins were also fixed in the mixture of ethanol and glacial acetic acid at 9 am. For preliminary examination to determine the stage of anthers, the Smirnov method (1968) was used. In the final variant, the catkins were stained by acetohematoxylin for 10–12 h. A total of 85 preparations were examined: 41 from the experimental region (9613 cells were analyzed) and 44 from the control region (7403 cells were analyzed). The results obtained were statistically processed using Stadia software.

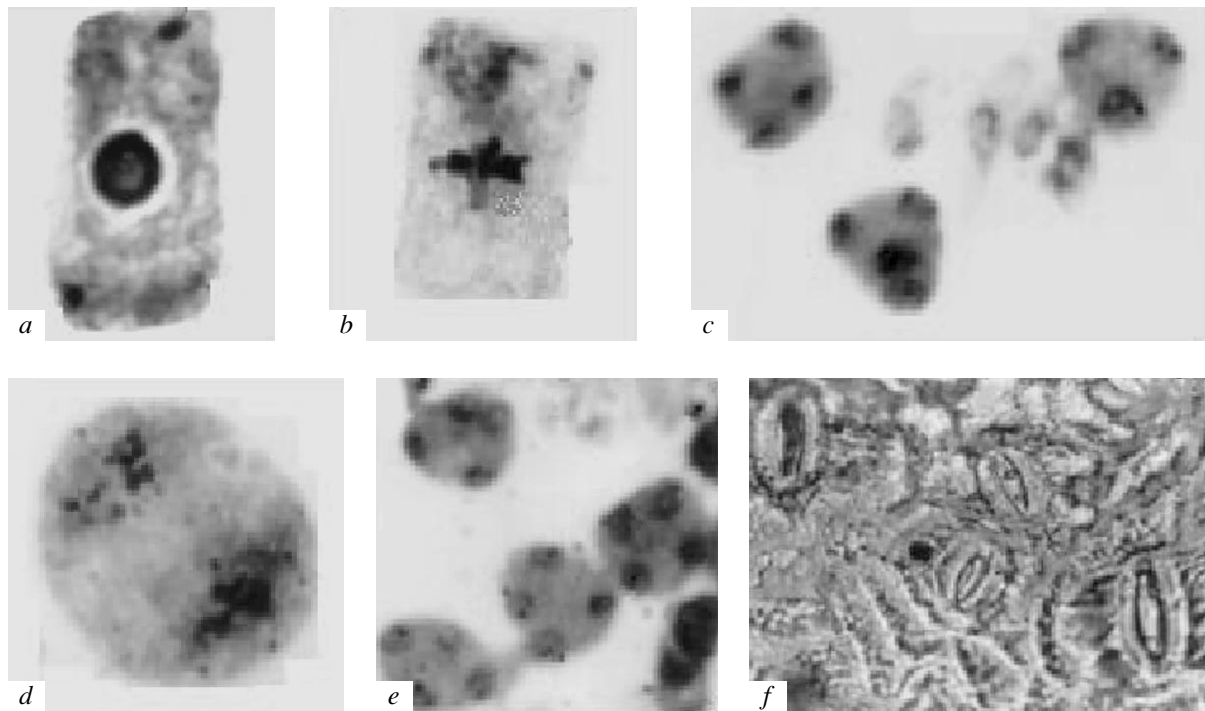
## RESULTS AND DISCUSSION

Cytogenetic analysis of weeping birch germlings (collected in 1998) has shown and statistical processing

has confirmed significant deviations from the normal course of mitosis in germlings of the seeds collected in the territory of Novovoronezh. The mitotic activity was higher than in the germlings from the ecologically pure zone ( $p < 0.05$ ): the mitotic index (MI) was  $9.83 \pm 0.59$  and  $8.60 \pm 0.22$ , respectively. Such a stimulating effect may be due to the influence of damaging factors, specifically irradiation at moderate doses (Li, 1963; Sanaev and Lazarenko, 1989). This phenomenon may be adaptive: apparently, in the area of weak irradiation, a maximum energy transfer takes place—an additive effect of the consumed dose and energy of  $\gamma$ -quants is expressed as an increased MI value. The stimulating effect of low doses of irradiation also follows from a certain decrease in the number of prophase, which may be considered an accelerated transition to the next phase of the cell cycle, and increased proportion of meta-, ana-, and telophases. The nucleolar activity in the experimental variant did not increase, as compared to that in the control.

The frequency of mitotic pathologies in germlings of the birch seeds collected in Novovoronezh was significantly higher than in the ecologically pure zone: its mean value in 1998 was  $9.47 \pm 0.53$  and  $3.41 \pm 0.24\%$ , respectively ( $p < 0.01$ ) and in 1999,  $9.63 \pm 0.69$  and  $3.02 \pm 0.14\%$ , respectively ( $p < 0.01$ ). Micronuclei (Fig. 1a), chromosome lagging, bridges, and chromatin fragmentation were among the most frequent mitotic anomalies in germlings of birch seeds collected in Novovoronezh. The latter could be a specific response of the cell to ionizing irradiation; most fragments were acentric and, hence, remained immobile and delayed in the course chromosome movement during metakinesis and disjunction toward the poles. Chromosome and chromatid bridges are due to chromosome fragmentation. Chromosome lagging is due, as a rule, to the influence of pathological conditions; at telophase, such chromosomes are either forced by a cytoplasmic constriction in one of the daughter nuclei, or form an additional nucleus (Alov, 1972). The frequency of micronuclei for the seeds collected in 1998 was  $0.24 \pm 0.18$  and  $2 \times 10^{-3} \pm 10^{-3}\%$  in the experiment and control, respectively ( $p < 0.01$ ) and in 1999,  $1.26 \pm 0.21$  and  $4 \times 10^{-3} \pm 10^{-3}\%$ , respectively ( $p < 0.01$ ). The presence of micronuclei in the cells suggests a chronic mutagenic effect (Il'inskikh *et al.*, 1988; Arutyunyan, 1990; Nersisyan, 1996). Since the micronuclear test is comparatively fast and simple, it is a convenient preliminary method for estimation of cytogenetic disturbances. However, it is advisable to use this test in combination with other indices, such as MI, nucleolar activity, and frequency and spectrum of mitotic pathologies, for estimation of possible mutagenic effects, since it does not permit the identification of the type of chromosome aberrations (Alov, 1972; Biotest..., 1993).

As regards the spectrum of mitotic abnormalities, chromosome lagging and, sometimes, bridges were mostly observed in germlings of the birch seeds from the ecologically pure territory, i.e., there were no stress-



Cytogenetic and anatomical abnormalities in weeping birch trees from Novovoronezh: (a) micronuclei at interphase of the cell cycle; (b) a residual nucleolus at metaphase; (c) a triad of nuclei; (d) elimination of chromatin regions in a microsporocyte at metaphase II; (e) cytomixis at the stage of nuclear tetrad; (f) closing cells of the stomata of different sizes.

induced chromosome aberrations. Note a high percentage of cells with so-called residual nucleoli at metaphase–telophase (Fig. 1b) in germlings of the birch seeds from the experimental variants: in 1998,  $7.56 \pm 0.49\%$  versus  $10^{-3} \pm 10^{-3}\%$  in the control ( $p < 0.01$ ) and in 1999,  $13.43 \pm 1.08\%$  versus none in the control, ( $p < 0.001$ ). This can be considered as a specific chromosome puffing reflecting the transcriptional activity in some areas upon disturbed DNA–protein interaction in the regions of nucleolar organizer under the influence of ionizing irradiation (Butorina *et al.*, 1997).

Cytoplasm vacuolization was also frequently observed in the experimental variant:  $8.03 \pm 0.15$  and  $5.79 \pm 0.44$  in 1998 and 1999, respectively (none in the control).

Thus, analysis of mitotic processes in germlings of the weeping birch seeds collected in Novovoronezh has shown significant changes in homeostatic processes in the plants, thus suggesting the presence of stress agents in the environment. Our results correlated with the data on a higher level of infantile diseases in that part of Novovoronezh, which was chosen for studies (the territory adjacent to the cooling pond).

In order to obtain a more comprehensive idea about the mechanisms underlying the adaptation of plants to unfavorable factors, we studied specific features of development of the male generative sphere in the chosen weeping birch trees. Being rather “sensitive,” the

stage of microsporogenesis is capable of responding to unfavorable environmental influences. Meiosis in the weeping birch trees from Novovoronezh was rather irregular. There were statistically significant differences in the total number of meiotic abnormalities between the experimental and control trees: 9.3 to 19.3% at the mean level  $13.62 \pm 1.67\%$  and 1.6 to 3.2 at the mean level  $2.42 \pm 0.29$ , respectively ( $p < 0.01$ ). Many uni- and polyvalents were present at prophase I in the experimental trees, although their counting was complicated because of small chromosome sizes. The highest number of abnormalities of the general type at prophase was 36.3 (tree no. 4) and the lowest, 2.4% (tree no. 3). No uni- and polyvalents were recorded in the birch trees in the ecologically pure zone. Deviations in prophase I in the experimental variant led to abnormalities at metaphase I: while bivalents were correctly distributed on the spindle equator, uni- and polyvalents disturbed this process and univalents were often scattered over the achromatic division spindle. Chromosome elimination and acceleration were other typical deviations at this stage of cell cycle. The total number of such deviations was rather high in the experimental variant: 8.6 in tree no. 3 to 32.8% in tree no. 1, at the mean value  $20.68 \pm 0.37$  and  $6.36 \pm 0.19\%$  in the experiment and control, respectively ( $p < 0.01$ ).

Simple and multiple bridges and chromosome lagging constituted other types of deviations from the normal course of meiosis in the weeping birch trees, which are quite abundant at anaphases I and II: the number of

Variations of sizes of closing stomatal cells of the leaf lower epidermis in experimental trees

Ordinal number of the tree	Stomata, %			Coefficient of variation $C_v$ , %
	small (11.25–24.15 $\mu\text{m}$ )	medium (24.15–37.05 $\mu\text{m}$ )	large (37.05–50.00 $\mu\text{m}$ )	
1	22	76	2	15.73
2	18	75	7	17.39
3	11	81	8	7.55
4	20	61	19	30.84
5	1	60	39	18.78

such deviations in experimental tree no. 1 was 25 and 40.9% at anaphase I and anaphase II, respectively, at the mean level (anaphase I)  $19.62 \pm 1.08$  and  $3.24 \pm 0.40$  in the experiment and control, respectively ( $p < 0.01$ ) and at anaphase II,  $19.50 \pm 0.67$  and  $8.90 \pm 0.15$ , respectively ( $p < 0.01$ ). Triads and polyads of nuclei at telophase II (Fig. 1c) were one more characteristic type of meiotic disturbances in the weeping birch trees from the experimental variant, which led to the formation of sporads with abnormal number of microspores (triads and polyads of spores); micronuclei were seen in some sporads. The highest level of disturbances at telophase II and stage of sporogenesis was recorded in the experimental tree no. 4: 9.3% (triads of nuclei and additional nuclei) and 12.8% (polyads, triads, and diads of spores; monads and tetrads with micronuclei), respectively. The differences in the level of anomalies between the experimental and control trees at the stage of tetragensis were statistically significant ( $p < 0.01$ ) and the total number of deviations was  $5.02 \pm 0.13$  and  $1.02 \pm 0.04$  in the experiment and control, respectively.

In addition to general types of abnormalities, specific deviations from the normal course of meiosis also occurred, such as multiple elimination of chromatin regions in the cell (Fig. 1d). This phenomenon was observed in birch trees from the experimental variant: if at all, elimination was seen in almost all microsporocytes. This could be due to the activity of some protective mechanisms of the cell against stress and, while eliminating these regions of chromosomes. Possibly, strongly modified under the influence of unfavorable factors), the microsporocytes “defended” themselves against some more serious consequences or even death. This phenomenon appears to be chromosome diminution, which appears to be adaptive for the weeping birch. It could be related to the activation of migrating genetic elements under unfavorable conditions, when some regions of the genome may be “switched off” and even eliminated to the cytoplasm.

Cytomixis is one more specific type of meiotic disturbance in the weeping birch (Fig. 1e). This type was

repeatedly noted as specific for the weeping birch (Woodworth, 1929; Brown and Al-Dawood, 1977; Butorina, 1985). In our experiments, cytomixis was observed as wide cytoplasmic channels, along which the exchange of cytoplasm and nuclear substance was realized between microsporocytes. Worthy of notice is the hypothesis, according to which cytomixis is a sequence of great differences in mechanical tension between adjacent cells, which are produced during the development of anthers (Tarkowska, 1966), as well as the hypothesis on meiosis synchronization due to the formation of intermeiocyte connections (Whelan, 1974). One more hypothesis is also interesting, according to which cytomixis is controlled by the genes that are responsible for pathological conditions in mother cells of the pollen. Transposable genetic elements, including viruses, which provide for the genome inconstancy, could have acted as such genes (Khesin, 1984). The genome hereditary variability based on transpositions of mobile genetic elements plays, as was noted above, an important role in adaptation. This means that the natures of cytomixis may differ, but a certain role of transposable genetic elements is not excluded.

Studies of meiosis in the weeping birch trees from the experimental plot allowed us to put forward the suggestion about their mixoploid nature due to a rather high frequency of uni- and polyvalents compared to the control, presence of microsporocytes of various sizes at all division phases, and a high degree of pollen inequality (the diameter of pollen grains in the experiment and control was 55.75 and 2.50  $\mu\text{m}$ , respectively). In order to test this suggestion, we determined the size of closing cells of the stomata of lower epidermis of their leaves. The results are given in the table.

It can be seen that the size of closing stomatal cells markedly varied in the experimental trees (Fig. 1f), and this can serve as an indirect evidence of the mixoploid nature (Burda and Shchepot'ev, 1973) of the trees we studied. Mixoploidy occurs rather frequently in leafy trees, since they are an evolutionary young group and have unstable genome. Mixoploidy may be related to

adaptation to unfavorable conditions: mixoploids have a rather stable genetic system due to a reserve of polyploid cells and, at the same time, are more plastic, since they are represented by the diploid cells as well. Apparently, the optimal combination of the cells of different ploidy provides for the most efficient gene functioning under specific environmental conditions.

The results we obtained suggest that the effect of the complex contamination of the environment in the 30 km zone surrounding the Novovoronezh nuclear power plant on weeping birch populations leads not only to significant deviations in development of the male generative sphere, but also to the appearance, in some trees, of adaptation to unfavorable conditions. A more distinct mixoploidy in the experimental trees is undoubtedly adaptive. Some other cytogenetic phenomena, such as chromosome diminution, may also be considered as mechanisms of adaptation; this means that the genome instability under extreme conditions provides greater possibilities for selection.

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NATIONAL POPULATION WORKSHOP:  
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**Evaluation of Developmental Stability and Cytogenetic Homeostasis in Populations of European Green Frogs (*Rana esculenta*-Complex) under Natural and Anthropogenic Conditions**

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**Abstract**—The status of amphibians in populations subjected to anthropogenic influence of various natures has been evaluated. We studied the effect of complex anthropogenic influences as well as chemical and radiation pollution. The status of the specimens in populations was evaluated by morphological (developmental stability as the level of fluctuating asymmetry) and cytogenetic (micronucleus test) methods. Disturbed developmental stability and cytogenetic homeostasis have been observed in populations affected by anthropogenic factors, which indicates the changed status of the organism.

*Key words:* developmental stability, cytogenetic homeostasis, fluctuating asymmetry, micronucleus test, chemical and radiation pollution.

Developmental stability is the capacity of the organism to form a genetically determined phenotype with the minimum level of ontogenetic disturbances (Zakharov, 1987). It depends on genetic and environmental factors. Nowadays the problem of environmental influence on developmental stability is the most relevant due to the increasing negative influence of anthropogenic factors on natural communities.

The influence of chemical pollution of the environment on developmental stability has been experimentally demonstrated for a number of species (Clarke and Ridsdill-Smith, 1990; Clarke, 1992; Graham *et al.*, 1993; Borisov *et al.*, 1997; Hardersen and Wratten, 1998; Chowdhury and Bromage, 2000).

Presently, developmental stability is a widely used index for evaluating the status of natural populations under the influence of various anthropogenic factors including chemical pollution (Pankakoski *et al.*, 1992; Chubinishvili, 1998a, b; Kozlov and Niemela, 1999; Zakharov *et al.*, 2000a, b; Eeva *et al.*, 2000) as well as ionizing and nonionizing radiation (Posledstviya..., 1996; Freeman *et al.*, 1999; Zakharov *et al.*, 2000b).

Fluctuating asymmetry—insignificant nondirectional deviations from perfect symmetry (Van Valen, 1962)—can serve as a measure of developmental stability. Even a faint environmental influence can deviate from the path of development from the genetically determined one, which gives rise to fluctuating asym-

metry. Hence, the evaluation of fluctuating asymmetry indicates how favorable the environment is for a given organism.

Cytogenetic homeostasis is another index of the organism status (Il'inskikh *et al.*, 1992). Chromosome and chromatid rearrangements such as asymmetric and incomplete exchange during mitosis give rise to acentric fragments, which can appear outside the daughter nucleus as micronuclei. The micronuclei can also contain complete chromosomes resulting from mutagen-induced damage of the division spindle. A micronucleus test was proposed to evaluate the level of cytogenetic damage (Evans *et al.*, 1959). A number of experimental publications demonstrated the relationship between the frequency of cells with micronuclei and concentration of a mutagen as well as exposure time (Hooftman and de Raat, 1982; Jaylet *et al.*, 1955; Van Hummelen *et al.*, 1989; Scarpatto *et al.*, 1990a; Zakhidov *et al.*, 1993). Applicability of this method for biomonitoring has been demonstrated (Scarpatto *et al.*, 1990b; Zhuleva and Dubinin, 1994).

Here, we present data obtained over several years of amphibian status evaluation in populations affected by various anthropogenic factors—complex anthropogenic influence as well as chemical and radiation pollution. Both the populations under chronic influence and those subjected to a single influence have been studied.

## MATERIALS AND METHODS

We studied representatives of the European green frog complex (*Rana ridibunda*, *R. esculenta*, and *R. lessonae*). A uniform pattern of characters was used to evaluate developmental stability in each of these species. Previously, we have shown a similar level of developmental stability under similar conditions in all three green frog species, including the hybrid form (Chubinishvili, 1997). This allows us to compare the data obtained for the species studied.

Developmental stability was evaluated in various regions of Russia with chemical and radiation pollution. The material was collected in the regions of Astrakhan, Samara, and Bryansk as well as Chuvash Republic.

**Astrakhan District.** Samples of marsh frog *R. ridibunda* were studied in the Astrakhan District. The populations living there suffer from complex anthropogenic influences. The material was collected in late September 1993 at four sites: 1, the left bank of the Volga River, 160 km upstream from Astrakhan; 2, the right bank of the Volga, 40 km downstream from Astrakhan (2a, the river bank and 2b, irrigation canal); and 3, the left bank of the Volga, 80 km downstream from Astrakhan.

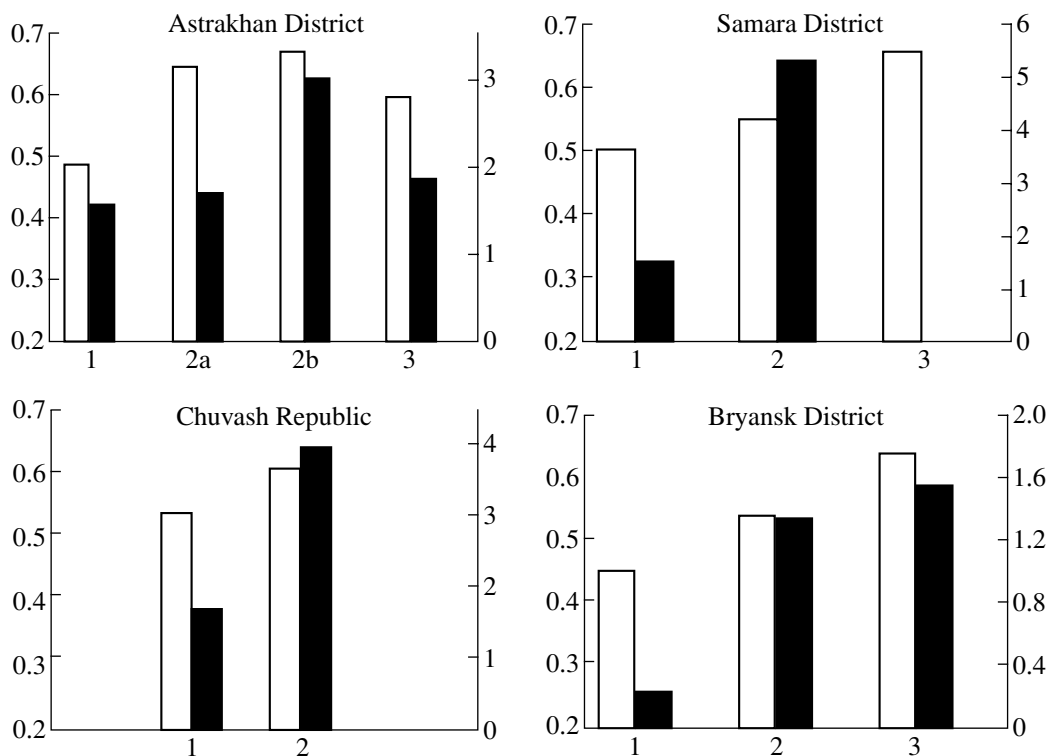
Site 1, located upstream from the main sources of pollution, was considered as a conventional control.

Site 2 is located in the region of the discharge of Astrakhan industrial waste and was selected to evaluate the situation in conditions of severe complex anthropogenic load. Site 3 was selected to evaluate the situation at a considerable distance from the main source of pollution—Astrakhan City—but is located in the region of active agricultural activity.

Sample volume for evaluation of developmental stability was 26, 27, 23, and 16 specimens in sites 1, 2, 2a, and 3, respectively. The sample volume for the cytogenetic test was 10, 8, 10, and 4 specimens for cytogenetic tests in sites 1, 2a, 2b, and 3, respectively.

**Samara District.** Developmental stability and cytogenetic homeostasis of marsh frog *R. ridibunda* were studied in the region of Chapaevsk (Samara District). Chemical weapons were produced there in the past. Presently, there are a number of chemical plants. The material was collected from May–June 1994 at three sites: 1, recreation zone at Ilmen Lake; 2, river region neighboring fertilizer plant; and 3, site of industrial waste discharge.

Site 1 was considered a conventional control. Although the site of material collection is only 2 km from the plant, due to the wind, it is not subject to active atmospheric pollution. The sample from site 2 was taken to evaluate the influence of chemical plant on the amphibians within close proximity, while the sample



Indices of developmental stability (frequency of asymmetric manifestation per character; left ordinate, (□)) and cytogenetic homeostasis (frequency of erythrocytes with micronuclei, %; right ordinate, (■)) in populations of European green frogs (*Rana esculenta* complex) from various regions of Russia; 1, conventional control collected in a region of relatively clean habitats; see text for explanations of other sites.

from site 3 was taken to evaluate the influence of industrial waste.

The sample volume for the evaluation of developmental stability was 14, 11, and 9 specimens at sites 1–3, respectively. The samples from sites 1 and 2 were used for the cytogenetic test; the sample volumes were 11 and 12 specimens, respectively.

*Chuvash Republic.* Developmental stability and cytogenetic homeostasis of marsh frog *R. ridibunda* was studied in the Chuvash Republic. After a railway accident in 1996, the studied territory was contaminated by phenol and petrochemicals. Hence, in this case, we studied the status of specimens subjected to single chemical pollution. The material was collected in September 1997 at two sites: 1, closed reservoir in Shumerlinsk Forestry; 2, Myslets Stream on the periphery of Myslets turn-out. Site 1 was considered a conventional control, while site 2 represented the railway accident region. The sample volume for the evaluation of the developmental stability was 20 and 7 specimens, respectively. The sample volume for the cytogenetic test was 10 and 6 specimens at sites 1 and 2, respectively.

*Bryansk District.* In the Bryansk District, we studied populations of pool frog *R. lessonae* and hybridogenic *R. esculenta*. The material was collected in 1993 and 1994 at sites with different X-radiation backgrounds.

In 1993, the samples from two sites were collected: site 2, Petryatinka, with  $0.021 \times 10^{-4}$ – $0.100 \times 10^{-4}$   $\mu\text{A}/\text{kg}$  exposure rate and site 3, Sennoe,  $0.057 \times 10^{-4}$ – $0.301 \times 10^{-4}$   $\mu\text{A}/\text{kg}$ . The sample volume for the evaluation of developmental stability was 26 *R. lessonae* and 33 *R. esculenta* specimens for Petryatinka and Sennoe, respectively.

In 1994, a sample was collected near “Bryanskii les” conservation—an ecologically safe region in Bryansk District (site 1). This sample was considered as a control. The sample volume for the morphogenetic test was 16 specimens (*R. esculenta*).

The cytogenetic test complemented the morphogenetic one. The sample volume for the cytogenetic test was 9, 6, and 9 specimens for “Bryanskii les,” Petryatinka, and Sennoe sites, respectively.

Developmental stability was evaluated by fluctuating asymmetry; three characteristic groups—color, skin, and osteology—were used for maximum accuracy (Zakharov *et al.*, 2000a). Thirteen characteristic were analyzed in total. Directed asymmetry and anti-symmetry has been revealed for neither of them, suggesting fluctuating asymmetry in these cases. The test for the characteristics’ mutual correlation was negative, which allows us to consider each character as an independent asymmetric property. The mean frequency of asymmetric manifestation per character (the number of asymmetric characters per specimen divided by the number of analyzed characters) was used as a measure of developmental stability. The significance of the dif-

ference between the samples was evaluated by Student’s *t* test (Sokal and Rohlf, 1981).

The micronucleus test was used to evaluate cytogenetic homeostasis. The micronuclei were accounted for in the peripheral blood erythrocytes using blood smears fixed in methanol for 10–15 min and stained by Giemsa dye for 10–12 min. The micronuclei were accounted for under microscope ( $\times 1000$ ). Two thousand erythrocytes were analyzed in each specimen. Student’s *t* test with Fisher’s  $\phi$ -transformation was used to compare frequencies of cells with micronuclei (Sokal and Rohlf, 1981).

Species were determined by polyacrylamide gel electrophoresis (Tunner, 1973; Mezhzherin and Peskov, 1992) and flow DNA cytometry (Vinogradov and Chubinishvili, 1999).

## RESULTS AND DISCUSSION

The evaluation of developmental stability in the populations from Astrakhan District yielded the following results. The mean frequency of asymmetric manifestation per character was  $0.49 \pm 0.03$ ,  $0.65 \pm 0.03$ ,  $0.67 \pm 0.03$ , and  $0.60 \pm 0.03$  at sites 1 (conventional control), 2a/2b, 2b, and 3, respectively (figure). The index of asymmetry at the conventional control site (1) significantly differed from those obtained in other sites ( $p < 0.01$ ). The differences between sites 2b and 3 were also significant ( $p < 0.05$ ).

The mean frequency of erythrocytes with micronuclei was 0.15, 1.68, 3.00, and 1.87‰ at sites 1, 2a, 2b, and 3, respectively (figure).

Significant differences ( $p < 0.001$ ) have been revealed between sites 1 and 2b as well as between 2a and 2b.

The data obtained indicate the least disturbance in the sample from the conventional control site (1), while the samples from sites under most severe anthropogenic influence (2a and 2b) were disturbed most. The level of disturbance at site 2b is higher compared to site 2a, apparently, due to the influence of an additional factor—fertilizers—on the specimens living in irrigation canals. Developmental stability of the specimen increases with the distance from the source of pollution (site 3) but remains low. Clearly, the river is slightly less polluted in site 3 due to self-purification; however, the anthropogenic influence remains heavy due to high agricultural activity along the river in the whole region studied.

The integrated index of developmental stability in the region of Chapaevsk in Samara District was 1,  $0.50 \pm 0.04$ ,  $0.55 \pm 0.04$ , and  $0.66 \pm 0.04$  at sites 1 (conventional control), 2 (in close vicinity to the plant), and 3 (industrial waste discharge site), respectively (figure). Comparison of the data obtained revealed a significant difference between sites 1 and 3 ( $p < 0.05$ ).

The micronucleus test yielded the following results: the frequency of cells with micronuclei was 1.5 and

5.33% at sites 1 and 2, respectively (figure); this difference was significant ( $p < 0.001$ ).

The status of the specimen from site 1 (conventional control) proved to be the safest among the studied samples. Even if there is a negative anthropogenic influence on this population (which can be completely avoided under urban conditions), it is insignificant.

Highly disturbed developmental stability and cytogenetic homeostasis have been revealed in the specimen from a plant-neighboring site 2, apparently, due to a negative industrial influence.

An extremely high level of developmental instability revealed in the amphibians from site 3 (industrial waste discharge site) indicates considerable status deviation of the specimen living in this region.

Amphibian populations from Chuvash Republic had the following mean frequency of asymmetric manifestation per character:  $0.54 \pm 0.07$  and  $0.61 \pm 0.02$  at sites 1 (conventional control) and 2 (the accident site), respectively (figure).

The micronucleus test yielded the following results: the mean frequency of erythrocytes with micronuclei was 1.67 and 3.59% at sites 1 and 2, respectively (figure). The difference between the data obtained in these two studied sites was significant ( $p < 0.001$ ).

Hence, the specimens under the influence of chemical pollution (site 2) featured both decreased developmental stability and disturbed cytogenetic homeostasis.

The following data were obtained for the populations from Bryansk District: the integrated index of developmental stability was  $0.45 \pm 0.03$ ,  $0.54 \pm 0.03$ , and  $0.64 \pm 0.03$  in sites 1 (control, "Bryanskii les" conservation), 2 (relatively low radiation pollution, Petryatinka), and 3 (relatively high radiation pollution, Sennoe), respectively (figure).

Comparison of the data obtained has revealed a significant difference between all samples ( $p < 0.05$ ).

The following data were obtained by micronucleus test: the frequency of erythrocytes with micronuclei was 0.22, 1.33, and 1.55% in sites 1 ("Bryanskii les" conservation), 2 (Petryatinka), and 3 (Sennoe), respectively (figure).

Significant differences have been revealed between the "Bryanskii les"—Petryatinka and "Bryanskii les"—Sennoe samples ( $p < 0.001$ ).

Hence, disturbances in developmental stability and cytogenetic homeostasis were most pronounced at the site with the highest radiation pollution.

The above data indicate coordinated changes in the indices of developmental stability and cytogenetic homeostasis. The populations subjected to the most unfavourable environmental factors featured both the highest morphological and the highest cytogenetic disturbances. The deviations were minimal at most safe territories.

The scale system is convenient for rough estimation of the organism status deviation from the norm. We

believe that the five-point system is quite informative; one point corresponds to conventionally normal background status of a population, while five points correspond to a critical status. The range of values between these thresholds is ranked in increasing order. The obtained data allow us to develop such a system. Developmental stability index ranged from 0.45 to 0.67 in the studied samples. This range was ranked into five conventional groups corresponding to the points. Thus, the proposed scale system for population status evaluation has the following form: developmental stability index below 0.50, from 0.50 to 0.55, from 0.55 to 0.60, from 0.60 to 0.65, and above 0.65 correspond to 1–5 points, respectively.

We believe that the proposed scale system adequately reflects the real situation. For instance, population status was described by five points corresponding to the critical condition in the most polluted regions (site 2 in Astrakhan District and site 3 in Samara District). The status of the population living at the site with the highest radiation pollution (Sennoe) in Bryansk District was described by four points, corresponding to a highly disturbed developmental stability. Four points correspond to the railway accident site in Chuvash Republic. Conventional control sites in Bryansk and Astrakhan Districts have one point (conventionally control status), while those in the Samara District and Chuvash Republic have two points (low level of disturbances).

Application of a complex approach evaluating the organism status using several indices (developmental stability and cytogenetic homeostasis, in this case) rather than one single index provides more objective information. Note the specific features of the used approaches. The indices of cytogenetic homeostasis reflect the organism's physiological response to stress; their normal values can be restored after the stress factor is eliminated. At the same time, the studied morphological properties of the organism are established during early ontogenesis and are preserved during the whole lifespan; they are a nonspecific response of the organism to stress. The morphological approach seems to be the most appropriate for ecological monitoring.

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## Ontogenetic Approach to Studying Natural Avian Populations

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**Abstract**—The asymmetry of digit folioidosis and the correlation of exterior characteristics have been studied in 20 passerine species. The level of the characteristic asymmetry depends on the phylopatry level and regularly increases in the species disposed to annual change of the nesting site. We assume that the revealed variability reflects interpopulation flow of genes. Age-, sex-, and biotope-related changes associated with urbanization have been revealed in the correlation of characteristics. Older birds have more pronounced correlation of characteristics; it is also more specific for males than females. Correlation of characteristics is less pronounced in the urban avian populations. These differences are interpreted in terms of stabilizing selection within intraspecific groups.

*Key words:* fluctuating asymmetry, correlation of morphological characteristics, passerine birds.

Population properties of individual bird growth and development are poorly explored, although such investigations at the level of species are common (also in Russia, e.g., Poznanin, 1979). Other ontogenetic properties studied at the level of populations include fluctuating asymmetry of bilateral structures and the correlation of morphological characteristics. These two aspects are the aim of the present investigation.

Investigation of fluctuating asymmetry is relevant since it is a manifestation of the specific form of variability—intraindividual—and one of the main indices of ontogenetic stability (homeostasis). In addition, the asymmetry level reflects the rate of genetic coadaptation, since it notably increases after hybridization at the level of genetically distinct intraspecific forms or closely related species (Mather, 1953; Zakharov, 1987; Biotest..., 1993).

Analysis of intraindividual differences in bilateral plumage characteristics performed by various researchers yielded the following results: a feedback between the asymmetry level of certain secondary sexual characteristics in males and their genetic quality have been proposed in swallows (*Hirundo rustica*) and zebra finch (Moller, 1990; Swaddle and Cuthill, 1994); the relationship between the asymmetry level and quality of the habitat where bilateral structures of blackbird (*Turdus merula*) nestlings were developed have been revealed (Moller, 1995); and different timing of autumn migration of willow-heron (*Phylloscopus trochilus*) with a different level of asymmetry have been shown (Rintamaki *et al.*, 1995).

This points to promising investigation of fluctuating asymmetry phenomenon in birds under various ecological aspects. Such investigation cannot be limited to plumage characteristics. Properties of leg folioidosis can

be quite informative in this case. The number of horny plates on the elements of the right and left legs is sometimes different, and this is a fluctuating type of asymmetry (Vengerov and Ivanova, 1995).

In terms of biometry, statistical correlation means the relationship between variables. In terms of biology correlation is the relationship between various characteristics in the organism as a whole. Two biological levels of mutual relations between parts of the organism are recognized. The first one is ontogenetic level when the relations are manifested during individual development; they are called correlations. The second level is phylogenetic when the relations are manifested during evolutionary transformation of the organism parts; they are called coordinations (Shmal'gauzen, 1982). There are grounds to believe that the correlations of definitive exterior characteristics revealed in the animals by statistical methods reflect ontogenetic correlations based on morphogenetic and ergonic mutual relations of the developing organism and body parts.

Investigation of correlations between four metric characteristics of passerines (*Passeriformes*) demonstrated a certain species-specificity related to flight performance (Terent'ev, 1970). Analysis of 18 exterior characteristics in the tree sparrow (*Passer montanus*) revealed the main correlating groups, the gender difference in the power of links between parts of the flying machine (Rostova and Chetverikova, 1981; Chetverikova, 1985).

Coordinated variability of the exterior characteristics in rock-pigeons (*Columba livia*) featured pronounced accordance between phenotypic and genotypic correlations (Johnson and Johnston, 1990). Hence, interpretation of the intraspecific group differences in the level and structure of correlations is valid

in terms of selection. Changed correlations between morphological characteristics have been revealed by comparison of wild and chase forms of mallard (*Anas platyrhynchos*) (Fokin *et al.*, 1990) as well as male common rosefinch (*Carpodacus erythrinus*) that survived or died during migration and wintering (Bjök-lund, 1992). Considering the high heritability of most metric properties, one can assume that phenotypic correlations reflect genotypic ones in birds.

## MATERIALS AND METHODS

The material was collected in the Voronezh Biosphere Reserve, which is located on the border between Voronezh and Lipetsk districts and partially in the city of Voronezh.

The fluctuating asymmetry of leg foliosis meristic characteristics was evaluated in 18 passerine species; correlations of exterior qualitative characteristics have been studied in 20 species of this order. The mean sample volume was 49 and 30 specimen for foliosis and exterior characteristics, respectively. The measurements and account of meristic characteristics were carried out on live birds released after ringing.

In passerines, the horny plates cover the anterior and posterior parts of the metatarsal bone and the upper part of digits. We analyzed the plate number variability only on the second, third, and fourth digits as the most unambiguously recognizable and accountable. The pattern of leg plating is similar in the studied species, which allows both intra- and intraspecific comparisons within the same community. The analyzed samples were composed of males and females of *ad* (two and more years) and *sad* (about a year) ages. Merging the gender groups is valid, since they have similar values and patterns of asymmetry. This resulted in fluctuating asymmetry indices for the whole population integrating manifestations for various generations; the mean number of asymmetric characteristics per specimen was used as a qualitative test (*Posledstviya...*, 1996). It is calculated as the total number of asymmetric characteristics for each specimen divided by the sample volume (including the specimen lacking the asymmetry). In our example, the top value of this index cannot exceed 3 for three studied characteristics.

The following metric characteristics were used to study the correlations: length (1) and width (2) of the wing; forearm length (3); width, length, and height of the beak (4–6); and length of sternal crest (7), tibia (8), metatarsal bone (9), third digit (10), tail (11), and second and fourth digits (12 and 13). The above characteristics were measured after Vinogradova *et al.* (1976) and Chemyakin (1988) within an accuracy of 0.1 mm.

Structural analysis of morphological characteristic correlations was performed using Terent'ev's correlation pleiads method (Terent'ev, 1959, 1960). The pleiads were revealed on a correlation cylinder using the

moving level method. All links of a given section level were plotted on a correlation ring by lines of different weight reflecting the power of their differentiation. In addition to the correlation pleiads method, the system of characteristics mutual relations can be studied by factorial analysis. One of its variants—the method of principal components—is used most. The factorial pleiads match or are similar to the correlation pleiads; however, they can be of independent interest in conditions of low-contrast of the relation levels (Shmidt, 1985). Previous parallel analysis of the correlation structure of avian morphological characteristics by the above two methods indeed demonstrated a high similarity of the results (Vengerov, 1997). This is why analysis was reduced to the method of Terent'ev's correlation pleiads in this work.

## RESULTS AND DISCUSSION

The minimum and maximum mean number of asymmetric characteristics per characteristic (MNAC) is 0.18 and 0.75, while the MNAC average for all 18 species is 0.46 (table). Let us divide the considered species into three groups corresponding to low (0.18–0.39), medium (0.4–0.59), and high (0.6–0.75) asymmetry. The first group includes nuthatch (*Sitta europaea*), song thrush (*Turdus musicus*), chaffinch (*Fringilla coelebs*), house sparrow (*Passer domesticus*), oxeye (*Parus major*), and yellowhammer (*Emberiza citrinella*); the third group includes robin (*Erithacus rubecola*), pied flycatcher (*Ficedula hypoleuca*), and siskin (*Spinus spinus*); the nine resting species form the largest second group. In most cases, the differences between species in the first and third groups are significant.

One can see that ecologically and taxonomically diverse species fall into the same groups; however, their similarity in an important property—the level of phylopatry—can be seen. We understand phylopatry as the return of the birds to their birthplace or former nesting site after wintering; habitat up to 5 km across is considered as a birthplace of small birds (Sokolov, 1991).

Two representatives of the group with minimum asymmetry—nuthatch and house sparrow—are sedentary or partially migrating birds. Their relation to the birth or nesting site is most pronounced among all considered species. Oxeye and (to a lesser extent) yellowhammer are similar in this respect. Chaffinch and song thrush are migratory birds; the first one features one of the highest levels of phylopatry (Sokolov, 1991). The data available on song thrush do not suffice for final conclusions; but it is at least not among the species with low phylopatry.

By contrast, in the case of the robins falling into the third group, the young birds do not return to their birthplace and the adult ones change their nesting site annually. Permanent exchange of nesting sites is specific for certain Fringillidae birds including the siskin with the

most pronounced asymmetry. Medium phylopatry is usually specific for pied flycatcher.

Hence, the obtained data indicate a trend to lower asymmetry in birds with tight territorial links. An important population index—the level of gene exchange between spatial groups—also depends on the extent of phylopatry. Each population is ecologically and genetically adapted to local conditions. Genetic adaptation assumes formation of balanced coadapted gene complexes providing for the formation of the best phenotypes under given conditions. Gene exchange between populations is important for the maintenance of species integrity as well as for diffusion of valuable hereditary changes appeared in one of populations. At the same time, hybridization of relatively different genotypes to a certain extent disturbs the coadaptation of gene complexes. Increased asymmetry of bilateral structures registered in the hybrids is one of reflections of the disturbance (Zakharov, 1987).

We believe that the increased asymmetry observed in birds with low phylopatry and the increased asymmetry noted in the hybrids are like phenomena differing only by manifestation power. The revealed increased asymmetry does not have a great absolute value and affects no vital structures; hence, it is not likely to disturb the adaptation of the specimen. This, apparently, provided for the appearance of this asymmetry while the asymmetry would be impossible for other characteristics highly canalized in development in the case of such close similarity of the crossing birds.

The uneven position of the species in the studied community relative to the limits of their ranges can be another cause of interspecific differences of birds by asymmetry level. Populations of the species located near the range boundaries are under less favorable ecological conditions, which can increase the asymmetry of characteristics. Three out of 18 considered species have a southern range limit near the investigation region (nuthatch, pied flycatcher, and siskin); these include species with the highest and lowest asymmetry levels. This alone should rule out the important role of population displacement relative to the range. In addition, nuthatch and pied flycatcher commonly nest to the south of the Voronezh Reserve and have multiple, stable, and productive populations in the conservation. As concerns siskin, the southern limit of its range during nesting goes exactly by the reserve territory; however, the specimen caught do not belong to the local population, since they were collected in the autumn, when multiple birds with more southern origins appear in the reserve.

Hence, the interspecific differences described in the avian asymmetry in the number of horny plates on the digits can be due to an uneven level of gene exchange between the populations.

At the interspecific level, the investigation of the asymmetry level in spatial groups living under different environmental conditions is particularly interesting.

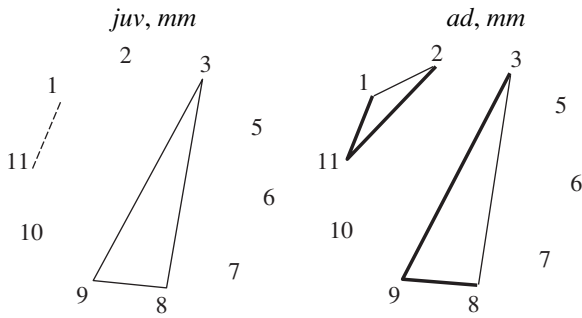
Asymmetry of foliodosis characteristics in various passerine species

Group, species	Number of specimen	Mean number of asymmetric characteristics
I		
<i>Sitta europaea</i>	33	0.18 ± 0.08
<i>Turdus philomelos</i>	48	0.21 ± 0.08
<i>Fringilla coelebs</i>	122	0.29 ± 0.05
<i>Passer domesticus</i>	66	0.32 ± 0.07
<i>Parus major</i>	87	0.38 ± 0.06
<i>Emberiza citrinella</i>	21	0.38 ± 0.13
II		
<i>Sylvia atricapilla</i>	42	0.42 ± 0.09
<i>Turdus merula</i>	43	0.42 ± 0.08
<i>Carduelis carduelis</i>	27	0.44 ± 0.12
<i>Sylvia borin</i>	41	0.49 ± 0.11
<i>Chloris chloris</i>	32	0.5 ± 0.13
<i>Coccothraustes coccothraustes</i>	44	0.52 ± 0.12
<i>Lanius collurio</i>	52	0.54 ± 0.08
<i>Riparia riparia</i>	65	0.57 ± 0.09
<i>Phylloscopus collybita</i>	37	0.57 ± 0.10
III		
<i>Erithacus rubecula</i>	33	0.61 ± 0.12
<i>Ficedula hypoleuca</i>	59	0.64 ± 0.10
<i>Spinus spinus</i>	24	0.75 ± 0.15

Such data are available for the house sparrow from the Voronezh Reserve and city of Voronezh. The integrated index—the mean number of asymmetry characteristics per specimen—was used. It equals  $0.32 \pm 0.07$  ( $n = 66$ ) for the reserve but is considerably higher in the city,  $0.62 \pm 0.08$  ( $n = 76$ ); the difference is reliable ( $p < 0.01$ ). One can conclude that the individual development of the house sparrow is more stable in the reserve compared to the city.

In addition to the fluctuating asymmetry, intra-individual variability can be studied using the example of the intraclutch variability of eggs. The formation of morphological, biochemical, and other characteristics of the egg are based solely on the female genotype; hence, eggs from one clutch can be considered homologous structures of the organism. Accordingly, the variability of these structures does not reflect individual genetic variability, rather it is a manifestation of one of the intra-individual variability forms (Vengerov, 1991).

Let us consider correlations of exterior characteristics using the age-, gender-, and biotope-related features as an example. Comparison of various age samples of chaffinch (birds of the current year (*juv*) and ones that are two or more years old (*ad*)) demonstrated an increased correlation of characteristics in pleiads of



**Fig. 1.** Correlation pleiads of exterior characteristics in age groups of chaffinch males; correlation coefficient  $r \geq 0.5$  (---);  $\geq 0.6$  (—); and  $\geq 0.7$  (—) (also in Fig. 3); 1–12, metric characteristics (see text for explanations; also in Figs. 3 and 4); *mm*, males and *ff*, females (also in Figs. 3 and 4).

older birds (Fig. 1). Most likely, this resulted from the elimination of individuals with disturbed proportions as indicated by the highest differences in the links between the most functionally important characteristics, namely, of the flying machine pleiad. The adult birds have already passed through stringent selection during migrations, interspecific competition for the territory, and pressure from predators.

This can be confirmed by the following observations. The size of wing and tail feathers changes during ecdysis (also in birds of *adultus* age). For instance, wing length was different in chaffinch males of this age after the first inspection and the second one 1–2 years later. Determining the stability of the correlations requires checking if the changes in the flying machine dimensions are correlated during ecdyses. This can be done by comparing the proportions of the characteristics in the same individuals after several years. The wing sharpness (width–length ratio) as well as the relative tail length (tail–wing length ratio) proved to be highly stable (Fig. 2), thus, indirectly confirming their strict genetic determination. The latter assumes nonrandom interpopulation variability by these characteris-

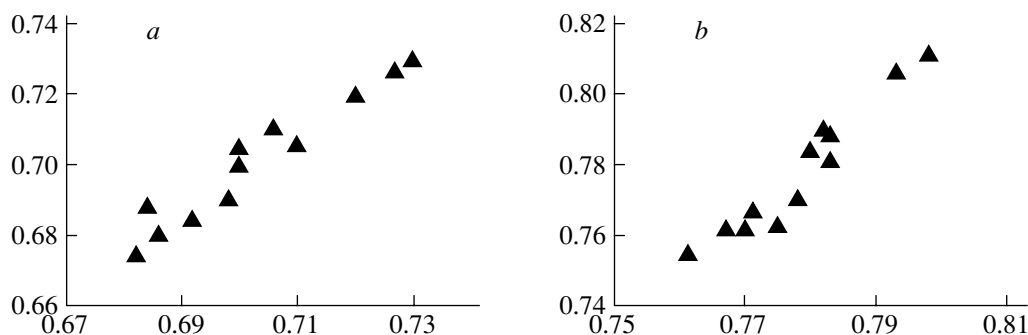
tics. The structure of this variability can be transformed in the populations under selective pressure as indicated by different levels of the correlations between the juvenile and adult individuals.

The correlations of avian morphological characteristics also include gender-related ones. More pronounced correlation of characteristics (Fig. 3) and sometimes their higher variability are observed in males of most studied species. This phenomenon can be considered as a particular case of sexual dimorphism manifested in many, but not all, species.

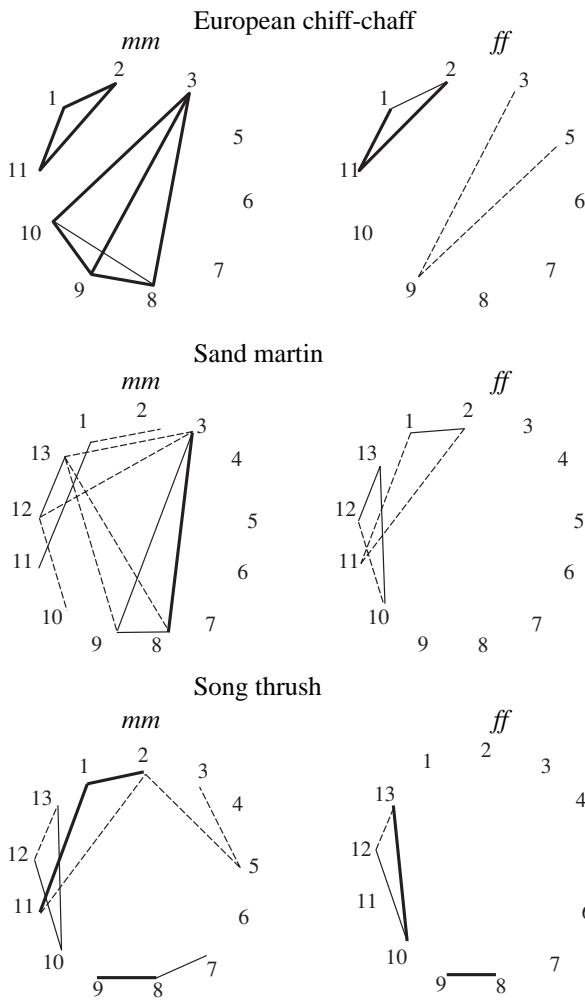
The differences considered are, apparently, due to ecological properties of the gender groups. The passerine males and females exercise unequal functions in hatching and training of the progeny; they can have different timing and pathway of migrations. These as well as other significant ecological particularities of genders inevitably influence the effect of stabilizing selection—the main factor controlling the extent of variability and correlation of characteristics.

Hence, the differences in the power and structure of correlation of characteristics between males and females of the considered passerine species are due to uneven activity of stabilizing selection in the gender groups. In the males stabilizing selection preserves the phenotypes in a more diverse dimensional range, providing their high morphological integrity.

The analyzed indices also feature biotopic differences related to urbanization. In these terms, we compared house sparrow samples from the city of Voronezh and the Voronezh Reserve. No significant differences in exterior characteristic variability were observed. However, there are significant differences in characteristic correlations: they are less pronounced in urban birds and have different pleiad structure (Fig. 4). Disintegration of morphological characteristics in house sparrows indicates decreased pressure of stabilizing selection in the urban territory. Only individuals with relatively high integrity of morphological structures are preserved by the selection in the even range of variability under reserve conditions, while individuals with disharmonious phenotypes can survive in the urban environ-



**Fig. 2.** Similarity between wing sharpness (*a*) and tail length (*b*) indices in 12 chaffinch males in the periods between ecdyses; ordinate: first inspection; abscissa: second inspection;  $r = 0.96, 0.98$ ;  $p < 0.01, 0.001$ .

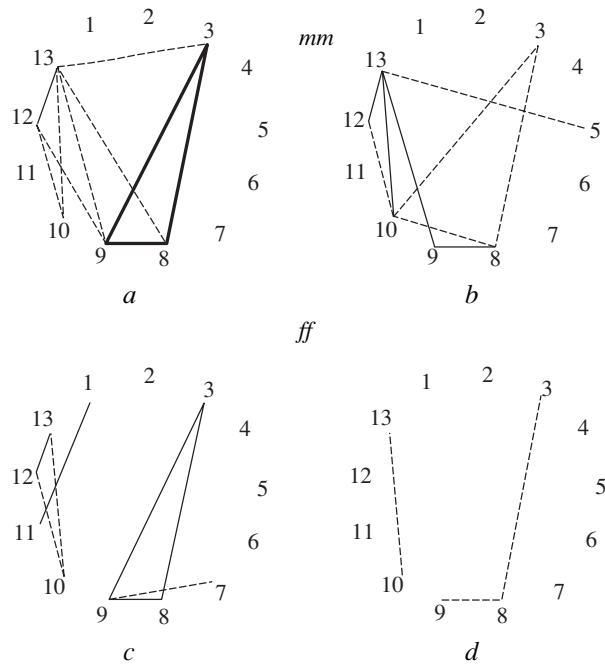


**Fig. 3.** Correlation pleiads of exterior characteristics in various species.

ment. Urban environmental conditions differ from those outside by many abiotic and biotic indices and altogether change the selective pressure.

Finally, fluctuating asymmetry shows no specific response to environmental factors. Diverse ecological analysis is required to determine the causes of its change in natural avian populations and the number of omnidirectional factors affecting the asymmetry should be minimized. Interspecific comparison is most efficient within the same community or relatively limited geographic space. They should be applied for single taxonomic groups with similar morphological structures.

Exchange of individuals (gene flow) between populations differs among avian species as indicated by results of ringing. Microevolutional processes in “closed” and “open” populations differ significantly (Mair, 1968), which makes clear the importance of the evaluation of gene exchange. The application of direct methods for this are limited in birds, while indirect evaluation based on ringing and repeated catching is



**Fig. 4.** Correlation pleiads of exterior characteristics in house sparrow from Voronezh Reserve (a and c) and City (b and d);  $r \geq 0.6$  (—);  $\geq 0.7$  (---); and  $\geq 0.8$  (-·-·-).

very labor-intensive. That is why the level of fluctuating asymmetry as the most accessible indirect index of gene flow can be quite promising for comparing populations of various species.

The intraspecific aspect of avian fluctuating asymmetry studies is presently reduced to the elucidation of two main relationships: asymmetry level– individual adaptation and asymmetry level–environmental quality for the population. The involved bio-topic differences are related to the environmental status. Such investigations are common for various taxonomic groups except birds (Zakharov *et al.*, 2000). The level of asymmetry proved to increase after chemical and radiation pollution as well as under the influence of other unfavorable factors. This index is valuable for bio-monitoring and conservation. Using birds as test species seems promising due to their wide diffusion, homoiothermy, and testing by vital methods. The most applicable indicator species include hollow-nesting birds that can be attracted to the desired places by artificial nests. The fledglings are studied in the course of development of the analyzed morphological structures.

Characteristics formed from similar primordia and related to executing certain functions (flying machine characteristics and leg elements) correlate most in passerines. This, coupled with the high heritability of avian quantitative indices, allow us to assume that mathematical correlations reflect the ontogenetic ones.

The correlations are variable at the intraspecific (intrapopulation) level. Age-, gender-, and biotope-related differences are formed as a result of stabilizing

selection. The older groups include individuals with proportionally formed structures while those with disturbed proportions are eliminated. Gender- and biotope-related differences are due to their ecological particularities and different tension of struggle for life.

The aforementioned properties allow us to use correlation analysis to study stabilizing selection in avian populations. Its advantages include higher sensitivity compared to analysis of single characteristics variability. Harmoniously formed phenotypes can be found in any (not necessarily modal) part of a certain characteristic distribution as has already been demonstrated for small mammals (Mezhzherin *et al.*, 1991). Hence, in the absence of differences between the compared samples in characteristic distributions, they are manifested in correlations of characteristic pairs or groups. Correlations of functionally linked characteristics directly interacting with the environment are preferable for analysis.

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## Estimate of Cytogenetic Homeostasis in Natural Populations of Some Small Murid Rodents

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**Abstract**—Cytogenetic homeostasis in natural populations under natural conditions and anthropogenic stress was estimated according to the frequency of chromosome aberrations in somatic cells for six species of small mammals. Cytogenetic homeostasis was disturbed under the stress effect of increased density during population cycles, at the ecological periphery, and in the case of environmental chemical and radiation contamination. Cytogenetic homeostasis disturbances were related to changes in other indices of homeostasis, such as developmental stability and immune status, suggesting the use of the cytogenetic approach for estimating the general state of individuals in natural populations.

*Key words:* cytogenetic homeostasis, chromosome aberrations, natural populations of rodents.

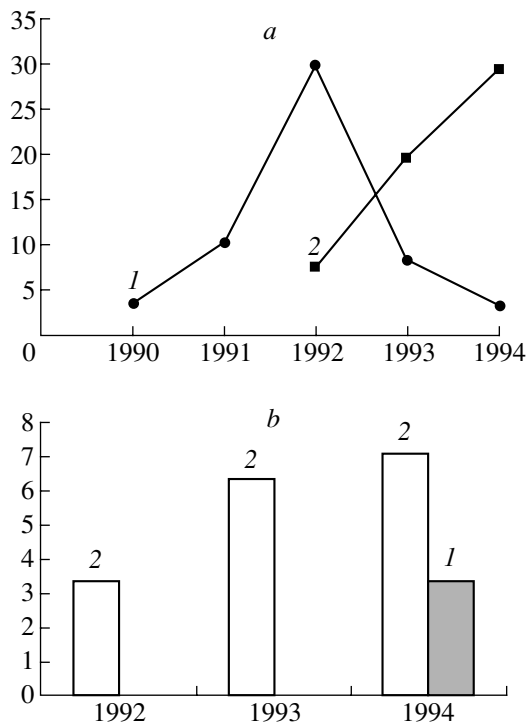
The use of the cytogenetic approach for studying the natural populations of mammals was related, above all, to investigations in the field of systematics, phylogenetics, and chromosome evolution (development of various concepts of chromosome speciation). The data on different types of cytogenetic rearrangements and their geographic variability in natural populations were of significant interest in microevolutionary studies. The number of studies, in which karyotyping and analysis of chromosome rearrangements are used, has decreased recently due to intense development in molecular methods of genome investigation.

At present, the use of the cytogenetic approach appears essential in estimating possible changes in the state of organisms in natural populations in space and time, since changes in the population ecological structure is considered as the most important factor of microevolution (Shvarts, 1980). When studying polymorphism of the natural populations in different parts of the range, especially in the ecological periphery, the changes in population state are described, which are induced by the stress effect of unusual environmental conditions. The populations located in the ecological periphery are called “natural laboratories,” where genetic experiments are carried out (Lewontin, 1978; Yablokov, 1987). These populations are an ecological avant-garde of the species, in which sharper changes in the selection direction and expression of evolutionary mechanisms can be awaited (Soule, 1973). Since their studies are important for understanding the evolutionary fate of the entire system of species population (Yablokov, 1987), an estimate of cytogenetic homeostasis or cytogenetic stability (Il'inskikh *et al.*, 1986; Broom and Johnson, 1993;

Biotest... , 1993; Kronenberg, 1994; Holmberg *et al.*, 1995; Zakharov *et al.*, 1996) represent an independent interest for characterization of the state of these populations. No less important is the cytogenetic estimate of possible changes in the state of an organism in time, above all, in the course of population cycles (Timofeev-Resovskii *et al.*, 1973, 1977; Shvarts, 1980; Yablokov, 1987). The data that overdensity leads to a distinct stress reaction (Christian, 1955, 1956; Thiesen *et al.*, 1971; Andrews *et al.*, 1972; Shilov, 1977) were of fundamental importance in studies on the dynamics of mammalian populations. Seredinin *et al.* were among the first to study the effect of stress on the cytogenetic stability of mammals. It was shown that emotional stress in inbred mice induced by overdensity markedly increased the number of abnormal cells (from 1.3% in the control to 4.8% in the experiment). When water voles taken from a natural population were kept under an increased density, the frequency of cells with both structural chromosome aberrations and altered number of chromosomes (polyploidy) increased (Skorova *et al.*, 1986). In response to stress, the frequency of crossover and spontaneous mutation increases (Belyaev and Borodin, 1982), DNA repair systems are inhibited (Borodin, 1987), and transposition of mobile genetic elements are induced (Vasil'eva *et al.*, 1997), which may lead to an acceleration in evolutionary processes (Imasheva, 1999). In this respect, the estimate of possible changes in cytogenetic homeostasis in the course of population cycles is of considerable interest.

In practice, analysis of cytogenetic homeostasis is essential for biomonitoring (Krysanov and Dmitriev, 1993; Zakharov, 1994; Zakharov *et al.*, 1996). The





**Fig. 1.** Dynamics of numbers (individuals per 100 traps/day) (a) and frequency of aberrant cells (%) (b) of mainland (1) and islet (2) populations of northern redbacked vole (*C. rutilus*) in Central Siberia in different years.

tasks of monitoring include an estimate of possible changes in the state of natural populations in nature (background monitoring) and study of the consequences of various anthropogenic influences, including chemical and radioactive contamination, etc. The use of the cytogenetic approach when solving these tasks looks promising both for characterization of the environmental genotoxicity and for estimation of the state of an organism.

Cytogenetic homeostasis was estimated through the study of the frequency of chromosome aberrations in the bone marrow cells at metaphase. The preparations of bone marrow metaphase chromosomes were made using the standard method. The frequency of chromosome aberrations was determined under an Amplival microscope at a magnification of 1000X. No less than 25 metaphase cells were examined for each animal. In order to reveal inter-population differences, we used the Cracksell-Wallis monofactorial nonparametric analysis of variance and, for paired comparison of the frequencies of aberrant cells, Student's *t*-test with Fisher's  $\phi$ -transformation of portions. The chromosome aberrations were classified and counted according to the standard recommendations. In this work we defined "true" aberrations as structural chromosome lesions (deletions, dicentrics, rings, and inversions) and quantitative chromosome aberrations (aneu- and polyploidy). Thus, the cells with at least one structural or

quantitative aberration were referred to the aberrant cells. Only hyperploid cells were considered as aneuploid, since chromosome loss could not be excluded during preparation.

#### ESTIMATE OF THE STATE OF POPULATIONS IN NATURE

We determined the frequency of chromosome aberrations at different population densities in the mainland and island populations of northern and common redbacked voles (*Clethrionomys rutilus* and *C. glareolus*) in Central Siberia (Dmitriev *et al.*, 1996, 1997).

For the island populations of both vole species, the negative effect of an increased density was already shown during the second year after the island colonization. This may be explained by the small area of this isolated territory. However, in the island population of the northern redbacked vole, cytogenetic homeostasis was disturbed (up to 7.11% aberrant cells in 1994) at a density close to that at the peak of density in a cyclic mainland population (Fig. 1). The differences in the frequency of aberrant cells in the years of high density were reliably higher ( $p < 0.05$ ) than during the period of low density. In the common redbacked vole, an increased frequency of the chromosome aberration (up to 7.9%) was observed at a relatively low density. This may be explained in that an increased density in both conspecific and heterospecific individuals may exert a stress effect (Zakharov *et al.*, 1991). An increased frequency of aberrant cells in the mainland population of common redbacked voles (more than 5%), whose numbers are always significantly lower than in a closely related species named the northern redbacked vole (Shvarts *et al.*, 1987), may be due to the fact that the region under study is on the periphery of the range of a given species. Aggravation of the physiological state, as determined from disturbed cytogenetic homeostasis, could be due to unfavorable biotic factors and insufficiency of suitable habitats, as well as competition with the closely related dominant species, the northern redbacked vole.

#### ESTIMATE OF THE STATE OF THE NATURAL POPULATION UNDER ANTHROPOGENIC INFLUENCE

Disturbances of cytogenetic homeostasis were studied under different kinds of anthropogenic influences (Dmitriev, 1997a, 1997b; Zakharov *et al.*, 1997, 2000).

*Novgorod District.* In order to estimate the consequences of chemical contamination, analyses of three rodent species were carried out near a chemical factory near the city of Novgorod: small mouse *Apodemus uralensis*, harvest mouse *Micromys minutus*, and common redbacked vole *Clethrionomys glareolus*. Since air pollutants from this factory represent the main danger for terrestrial ecosystems, the materials were collected in some points of a transect corresponding to the main

direction of winds in the region under study: from southwest to northeast. Point 1 is located in the factory and points 2–4 are located at distances of 2 (dumping ground), 9, and 20 km from the factory, respectively. Point 4 was used as a conditional control for the characterization of the environmental background, since it was the most remote from the factory.

In all three species, the frequency of aberrant cells increased as the distance from the factory decreased (Fig. 2). The frequency of aberrant cells in the small mouse in the region with the highest contamination was 11.3 and 12% and at a significant distance (point 4), 4.6%. The differences between the samples from points 2 and 4 were statistically significant ( $p < 0.02$ ). In the harvest mouse, the frequency of aberrant cells in points 1 and 2 was 18.5 and 15.5%, respectively, and in point 4, 5.0%. The differences between the samples from points 1 and 2, and 4 were statistically significant ( $p < 0.01$ ). The cytogenetic defects found near the source of the contamination suggest the presence of strong chemical clastogens. Significant aberrations, such as chromosome deletions, were found only in those animals that were caught near the factory. In addition, in the harvest mice from the factory area, cells with structural chromosome defects, dicentrics, were found.

*Lower Volga flow (near Astrakhan')*. In the Lower Volga flow, the most abundant species of murid rodents, house mouse *Mus musculus*, was analyzed (Fig. 3). The materials were collected at three sites: site 1—a forest-steppe plot on the left bank of the Volga, 160 km upstream from Astrakhan'; site 2—cultivated fields on the right bank of the Volga, 40 km downstream from Astrakhan' (in the region of Ikryanoe settlement); and site 3—cultivated fields on the left bank of the Volga, 60 km downstream from Astrakhan'. Sites 2 and 3 were chosen for estimating the situation in zones of intense anthropogenic influence, and site 1 was selected as a conditional control. The frequency of aberrant cells was the highest in the zone of maximum contamination (6.62% in site 2) and the lowest at a significant distance from Astrakhan' (1.72% in site 1). The differences in the frequency of aberrant cells in mice from sites 1 and 2 are statistically significant ( $p < 0.05$ ), and those between mice from sites 1 and 3 are close to statistically significant ( $p < 0.05$ ). Even at a great distance from Astrakhan' (site 3), the frequency of chromosome aberrations remained sufficiently high (5.75%), apparently due to intense agricultural activity in this region.

*Middle Volga flow (near Chapaevsk)*. In the Middle Volga flow, the common red-backed vole was analyzed. The materials were collected at three sites: site 1a—a forest strip near a chemical fertilizer plant, where chemical weapons had been produced in the past (Chapaevsk); site 1b—a forest strip near the Polymer plant (Chapaevsk), and site 2—a forest strip near the village of Yasnaya Polyana (10 km from Chapaevsk). Sites 1a and 1b were chosen for estimating the situation

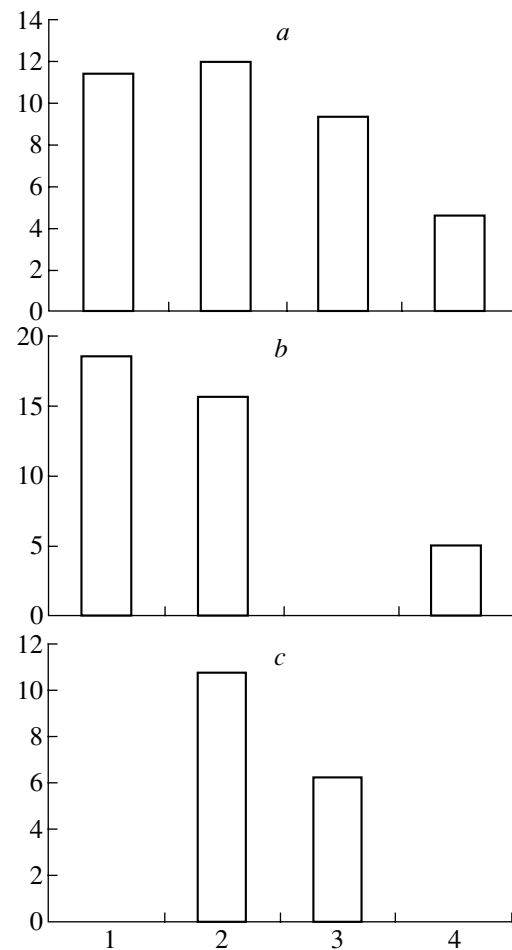
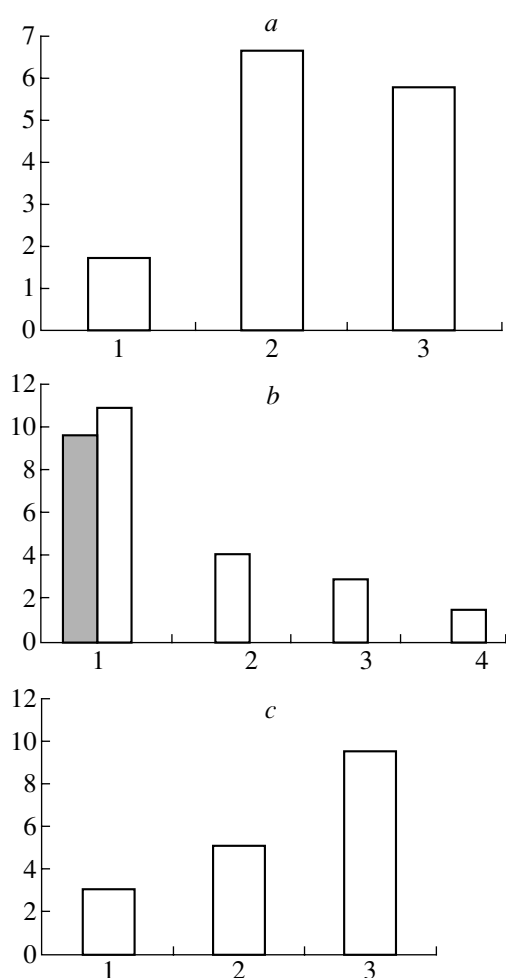


Fig. 2. Frequency of aberrant cells (ordinate, %) in samples (abscissa) of rodents from different sites of the Novgorod District: (a) *Apodemus uralensis*, (b) *Micromys minutus*, (c) *Clethrionomys glareolus*.

in zones of intense chemical contamination, and site 2 was selected as a conditional control. Similar high frequencies of aberrant cells were recorded in the region of intense chemical contamination (9.04 and 9.63% in sites 1a and 1b, respectively), as compared to the control (2.58% in site 2) (Fig. 4a). The differences in the frequency of aberrant cells in the voles from sites 1a, 1b, and 2 are statistically significant:  $p < 0.02$  and  $p < 0.01$ , respectively.

*Chuvashiya (Myslets crossing)*. In order to estimate the consequences of chemical contamination, two rodents, the common red-backed vole and the small mouse, were analyzed near the scene of a railway accident near Myslets crossing (Chuvashiya), where the soil, air, and surface and groundwaters were strongly contaminated by liquid phenol, petrochemicals, and dioxins. The materials were collected at several sites located at similar distances from the scene of the accident in the northwestern direction (the main wind direction at the moment of accident): site 1—near the site of the accident, along the Myslets River, in the region of



**Fig. 3.** Frequency of aberrant cells (ordinate, %) in samples (abscissa) of the house mouse *Mus musculus* from different sites of the Astrakhan' District (a), common red-backed vole *Clethrionomys glareolus* (□) and the small forest mouse *Apodemus uralensis* (■) from different sites of Chuvashiya (b), and common red-backed vole *Clethrionomys glareolus* from different sites of the Bryansk District (c).

Myslets crossing; site 2—near the village of Ryabinushka (1 km from the scene of the accident), site 3—near Chertagany village (2.5 km from the scene of the accident), and site 4—islet forest between Volga and Sanachili villages (20 km from the scene of the accident). Site 1 serves for characterizing the situation at the scene of the accident, sites 2 and 3 were chosen for estimating the quality of the environment near and at a certain distance from the scene of the accident, and site 4 was selected as a conditional control.

The highest frequency of chromosome aberrations in the common red-backed vole was recorded near the scene of the accident, at site 1. The frequency decreased with the distance from the accident site to achieve a minimum at the site of the conditional control (site 4). The differences between sites 1–3 and 4 are statistically significant at  $p < 0.05$ . Cytogenetic homeostasis and mutagenic activity were also estimated according to the

frequency of chromosome aberration for the small mouse near the scene of the accident near Myslets crossing (site 1) (Fig. 3b). The frequency of aberrant cells significantly exceeded the background level recorded for this species in some other regions.

**Moscow.** In Moscow, one of the most abundant species of murid rodents, the striped field mouse *Apodemus agrarius*, was analyzed (Fig. 5). The materials were collected at some sites located in different regions of the city: site 1—forest park in the Krylatskoe region, site 2—a margin of forest park 50 m from Leninskii prospekt (region of Troparevo), site 3—near Aviamotornaya ulitsa, and site 4—near an oil refinery in the Kapotnya region. The frequency of aberrant cells in all studied samples from different regions of Moscow proved to be higher than in the usually recorded level of chromosome aberrations in the conditional control sites ( $p < 0.05$ ).

**Bryansk District.** Studies of the effects of low doses of ionizing irradiation for the estimation of the potential hazard are urgent, since large territories of Russia were to some extent contaminated by radionuclides as a result of the accident at the Chernobyl' Nuclear Power Plant. It was of interest to carry out such estimates directly in regions with different levels of contamination, in order to reveal the possible radiation effects (Krysanov *et al.*, 1996; Dmitriev, 1997c, 1997d). Samples of common red-backed voles were taken in three sites: site 1—the village of Zelenyi Gai, Klimovskii region; site 2—the village of Petryatinka, Zlynkovskii region; site 3—the village of Krasnyi Kamen', Zlynkovskii region. The dose power was within the background limits at site 1 ( $0.014 \times 10^{-4}$  ( $0.012 \times 10^{-4}$ – $0.018 \times 10^{-4}$ ))  $\mu\text{A}/\text{kg}$ , exceeded the norm at site 2 ( $0.043 \times 10^{-4}$  ( $0.022 \times 10^{-4}$ – $0.1 \times 10^{-4}$ ))  $\mu\text{A}/\text{kg}$ , and significantly exceeded the norm at site 3 ( $0.129 \times 10^{-4}$  ( $0.05 \times 10^{-4}$ – $0.25 \times 10^{-4}$ ))  $\mu\text{A}/\text{kg}$ . The frequency of aberrant cells was the highest in the region of maximum radiation contamination (9.75%) and the lowest at the background level of radiation (3.05%) (Fig. 3c). The differences in the frequencies of aberrant cells in the voles from sites 1 and 3 are statistically significant,  $p < 0.01$ . Chromosome breaks and exchange chromosome aberrations were practically completely absent in animals from regions with increased radiation contamination, which is explained by the influence of a relatively low radiation contamination.

Thus, an intimate relationship has been shown between the increased frequencies of aberrant cells and occurrence in territories with an increased level of radiation contamination. This effect was not related directly to individual levels of  $^{137}\text{Cs}$  accumulation by rodents (Krysanov *et al.*, 1996). Note that quite evident effects may be expressed as changes in the state of animals under conditions of long-term occurrence in the regions with an increased radiation background and unequal distribution of radionuclides (Zakharov *et al.*, 1996; Baraboi and Oleinik, 1999). This may be due to dis-

turbed genetic coadaptation or genome stability (Gileva *et al.*, 1996; Bashlykova, 2000). As a result, the frequencies of aberrant cells and the proportion of individuals with chromosomal instability increase.

In all of the aforementioned examples, the increased frequency of the aberrant cells and markedly disturbed cytogenetic homeostasis was observed at sites with a maximum anthropogenic load, while the degree of deviation from the conditional control increased with the load. Deletions of the chromatid type was the dominant type of fixed nonbalanced chromosome aberration in almost all studied populations of rodents, while the proportion of other defects was significantly lower.

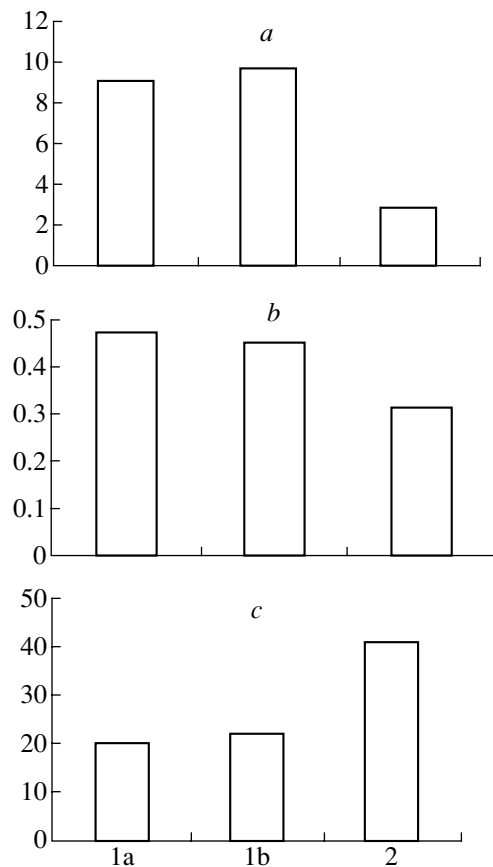
The frequencies of cells with significant structural defects of chromosomes were much lower: lesions of both chromatids (deletions and chromosome gaps), multiple chromosome aberrations, and aberrations of the exchange type (dicentric and rings). The latter were only recorded in the animals subjected to a strong anthropogenic load. All studied populations of murid rodents had in common that serious chromosome defects were only recorded in populations where the effects of a stress factor were at maximum.

No statistically significant increase in the frequency of aneuploid (hyperploid) cells, as compared to the control, were found in any of the studied populations, although they were the most frequent in the populations most susceptible to stress factors.

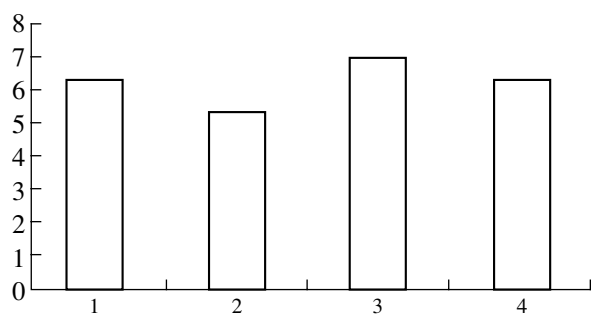
Interspecific comparison of the level of spontaneous mutagenesis and the background level of chromosome aberrations in most studied populations of small murid rodents in conditionally "pure" regions has shown that the frequency of aberrant cells did not exceed 2–3%. Analysis of published data (Salyaev, 1974; McBee *et al.*, 1987; Gileva *et al.*, 1992, 1993, 1996, 1999; Kryukov *et al.*, 1993, 1995; Kosareva, 1995; Gileva, 1997; Nokhrin, 1999) and our results suggest, with a certain degree of discretion, that this level is a threshold when determining the background level of cells with chromosome aberrations in populations of various murid rodents.

The main range of deviations in the frequencies of aberrant cells from the background level is within 12%, with the sole exception of a population of harvest mice from the territory of the Novgorod chemical plant, in which the range was up to 18.5%.

Thus, analysis of the background level of cytogenetic defects and of the degree of deviations has shown that diverse effects induced the same reactions, thus suggesting unidirectionality of these defects. This reaction appears not to depend on the kind of effect and, despite some interspecific differences, proved to be similar in different species. Comparative analysis of our results and the published data makes it possible to rank the observed changes in the frequency of aberrant cells according to a five-



**Fig. 4.** Indices of cytogenetic homeostasis (frequency of aberrant cells, %) (a), morphogenetic homeostasis (mean frequency of asymmetric expression per character) (b), and immune status (proliferative activity of splenocytes, 1000 cpm) (c) in samples of the common red-backed vole *Clethrionomys glareolus* from different sites in the Samara District: (1a) near the plant, (1b) near the plant, (2) in 10 km from the town.



**Fig. 5.** Frequency of aberrant cells in samples of striped field mice *Apodemus agrarius* taken from different sites in Moscow, %.

point scale: point 1—up to 3%, point 2—up to 6%, point 3—up to 9%, point 4—up to a3%, and point 5—more than 12% of aberrant cells, including quantitative and qualitative defects.

RELATIONS BETWEEN INDICES  
OF CYTOGENETIC HOMEOSTASIS  
AND OTHER CHARACTERISTICS  
OF THE STATE OF ANIMALS

Comparison of the data obtained using the cytogenetic method with the results obtained using other approaches (Zakharov *et al.*, 1996; Dmitriev, 1997d) was an important aspect of the present studies. In order to answer the question whether the described changes in cytogenetic homeostasis are related to those in the general state of animals under stress, it is essential to carry out parallel analysis using other approaches characterizing different aspects of functioning. Such analysis was carried out for the common red-backed vole on the Middle Volga flow (near Chapaevsk). In addition to the cytogenetic analysis, indices were used that characterize morphogenetic homeostasis and immune status. Fluctuating asymmetry (nondirected changes in the character value on different body sides) was used as the main morphogenetic characteristic of developmental stability (Zakharov, 1987, 1994; Developmental homeostasis..., 1997). The index of developmental stability that was used (mean frequency of asymptotic expression per character) demonstrated coordinated changes in a sequence of studied points as the level of territory contamination decreased (Fig. 4b). The data obtained suggest a disturbed stability in the two sites with the highest contamination.

In order to characterize the immune status several tests were used: functional activity of macrophages—activity of the enzyme 5'-nucleotidase, spontaneous proliferation of leucocytes—splenocytes, reaction of blast transformation of T- and B-cells with T- and B-mitogens, concanavallin A and liposaccharide, respectively (Pronin *et al.*, 1993, 1996a, 1996b). One of them is given here as an example: spontaneous proliferative activity of splenocytes, which reflects the reaction of lymphocytes to environmental factors. Spontaneous proliferative activity was inhibited under the influence of immunodepressive factors. The ecological conditions in the sites with the highest chemical contamination exert an immunodepressive effect on the spontaneous proliferative potential of the splenocytes of murid rodents (Fig. 4c). The results of estimating the state of animals obtained when using different approaches (cytogenetic, morphological, and immunological) coincide on the whole (Fig. 4). Hence, the cytogenetic approach may be used as an integral measure of disturbances, which characterizes not only the cytogenetic homeostasis, but also the general state of the organism.

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“ONTOGENESIS AND POPULATION”

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## Morphological Polyvariance of Ontogeny in Natural Plant Populations

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**Abstract**—The main patterns of expression of the morphological polyvariance of plants are discussed: diversity of biormorphs, pathways of ontogeny, and disturbances of morphogenesis. The diversity of biormorphs of tap root plants in different ecological conditions has been analyzed in detail. Promising directions of future studies have been formulated.

*Key words:* plant populations, plant ontogeny, polyvariance of ontogeny, morphogenesis, biormorphs.

Nowadays, there are two approaches to understanding development. Ontogenesis is often restricted to embryogenesis. In such a narrow sense, the subsequent periods of life remain beyond the ontogeny and constitute the postontogenetic (postembryonic or postnatal) period. This is the traditional approach based on the Haeckel concept “Ontogeny is a history of embryo development.” Another, wider approach includes in the ontogeny both development (embryogenesis) and subsequent life until the completion of development as a result of aging and death.

The differences in approaches of different researchers appear to be determined by specific features of the objects and different principles of their organization: unitary in most animals and modular in plants, fungi, and some animals, such as sponges, hydroids, corals, and pearlwrorts. The modular organisms are characterized by the polar and metameric body structure and unlimited growth (Zaugolnova *et al.*, 1988; Bigon *et al.*, 1989).

Rabotnov (1950) proposed periodization of plant ontogeny on the basis of the wider approach, which was later substantially complemented and developed by Uranov (1975) and his students (Tsenopopulyatsii..., 1976; *Ontogeneticheskii atlas...*, 1997, 2000; Gatsuk *et al.*, 1980). Four periods and 12 ontogenetic states are now distinguished in the plant ontogeny. The periodization is based on the concept of biological age as the organism’s own time. The changes in ontogeny are an expression of development in time. Hence, any ontogenetic state can be considered as a measure of biological time. It is characterized by a set of morphological characteristic-markers, not inherent in earlier developmental stages, and the disappearance (full or partial) of the previous characters. Thus, the ontogenetic states can be considered the key moments of development characterized by specific features of morphogenesis, certain ratios of *de novo* formation and dying, morphobiologi-

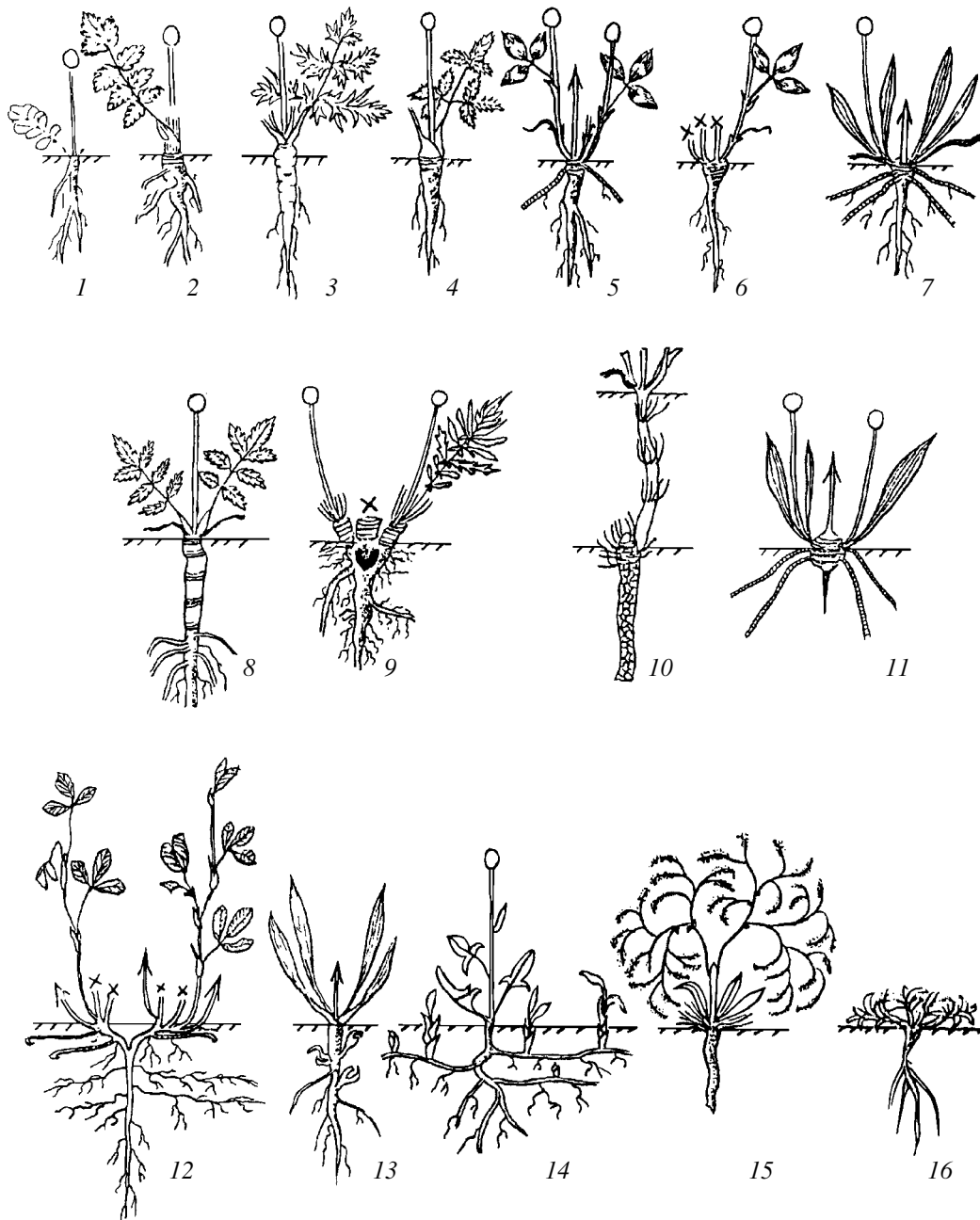
cal markers, and specific physiologobiochemical processes. The efficiency of this approach has been confirmed by the results studying the ontogenies of more than 500 seed plants, more than 20 ferns (Shorina, 1981), and two lichens (Suetina and Zhukova, 1997; Mikhailova and Vorobeichik, 1999).

In the 1960s–1970s, different modifications of ontogenetic states were described (Sabinin, 1963; Tsenopopulyatsii..., 1976; Zaugolnova *et al.*, 1988). This phenomenon was called polyvariance of development (polyvariance of ontogeny). The polyvariance of ontogeny is realized at both the organismic (individuals and ramets) and suborganismic levels (polyvariance of the development of organs characterized by morphogenesis or partial ontogeny). Classification of different expressions of the polyvariance of ontogeny has been proposed and two supertypes are distinguished: structural (types: dimensional, morphological, and reproduction methods) and dynamic (types: rhythmological and development rates) (Zhukova and Komarov, 1990; Zhukova, 1995).

The aim of the present study was to analyze the morphological polyvariance of ontogeny in natural populations of plants of different biormorphs. This problem has been extensively studied on tree (Chistyakova, 1978; Istomina and Bogomolova, 1991) and herbaceous plants (Tsenopopulyatsii..., 1976; Zaugolnova *et al.*, 1988; Zhukova, 1995; Nukhimovskii, 1997).

The succession of life forms is observed in many plants during development. Even the trees begin their life and single-shoot herbaceous plants (germlings and, more rarely, juveniles), while lignified shoots appear only during the second–third year. This is a typical course of ontogeny, during which morphogenetic programs of organs are switched on at certain stages and, as a result, the biormorph as a whole develops. At the same time, the older organs die, which leads to particu-





**Fig. 1.** Diversity of variants of the tap root biomorph of flower plants: (1) annual tap root biomorph, winter-ress *Barbarea vulgaris* R.Br.; (2) biannual or annual/biannual tap root biomorph, parsnip *Pastinaca sativa* L.; (3) biannual tap root biomorph with storing principal root, carrot *Daucus carota* L.; (4) perennial rhizomatous-tap-root biomorph with a compact zone of shortened internodes preserving the principal shoot and principal root until the end of ontogeny, wild parsley *Anthriscus sylvestris* L.; (5) "middle-leaved" rhizomatous-tap-root biomorph preserving the principal rosette shoot and forming generative shoots of the second and higher orders, red clover *Trifolium pratense* L.; (6) perennial tap root biomorph with a sympodial system of substituting shoots preserving the principal root, red clover *Trifolium pratense* L.; (7) perennial rhizomatous-raceme-tap-root biomorph with a mixed type of root system and preserving principal shoot, ribwort *Plantago lanceolata* L.; (8) perennial tap root biomorph with single-headed caudex, *Seseli libanotis* (L.) Koch.; (9) perennial tap root biomorph with multi-headed caudex, greater knapweed *Centaurea scabiosa* L.; (10) perennial tap root caudex biomorph with upper-rosette shoots, field eryngo *Eryngium campestre* L.; (11) perennial rhizomatous-raceme-tap biomorph with early dying principal root and preserving the principal shoot, ribwort "Plantago lanceolata" L.; (12) polycentric long-rhizomatous tap root biomorph, sickle medick *Medicago falcata* L.; (13) perennial tap root biomorph forming root offshoots on the principal root, ribwort *P. lanceolata* L.; (14) perennial polycentric tap root biomorph forming root offshoots on lateral roots, sheep's sorrel *Rumex acetosella* L.; (15) tap root biomorph "babies' breath," *Gypsophila paniculata* L.; (16) radial-flat pulvinate tap root biomorph, cushion pink *Silena acaulis* L.

lation. In this case, the morphogenetic processes take place during the life of an individual ramet.

Succession of life forms or appearance of different biomorphs in one or different plant populations is an extreme expression of morphological polyvariance. The life form, or biomorph, is an external appearance of the adult plant (Serebryakov, 1964), the biomorphs of the small-leaved lime *Tilia cordata* Mill. described by Chistyakova (1978) being a good example. Chistyakova distinguished single-trunk, few-trunk, multi-trunk, and grove-forming trees, tree-shrub, and facultative elfin wood.

Many examples of similar diversity in the populations of herbaceous plants have been described in both morphological and populational studies. The tap root herbaceous polycarpous plants were most extensively studied in this respect. We succeeded to describe 16 variants of this life form on the basis of our own materials and published data (Fig. 1).

The diversity of variants of the tap root biomorph of flower plants looks as follows.

Species	Biomorphs (see Fig. 1)
<i>Centaurea scabiosa</i>	4, 8, 9, 10
<i>Chelidonium majus</i>	2, 4
<i>Helichrysum arenarium</i>	1, 2, 6, 12
<i>Medicago falcata</i>	4, 6, 9, 10, 12
<i>Pastinaca sativa</i>	1, 2, 3, 6, 10
<i>Pimpinella saxifraga</i>	4, 5, 6, 9
<i>Plantago lanceolata</i>	4, 7, 11, 13, 14
<i>P. major</i>	5, 7, 9, 10, 11
<i>Rumex acetosella</i>	4, 6, 10, 13, 14
<i>Seseli libanotis</i>	4, 8, 10
<i>Scabiosa ochroleuca</i>	4, 6, 8, 9
<i>Taraxacum officinale</i>	4, 6, 8, 9, 10, 13, 14
<i>Trifolium montanum</i>	6, 8, 9, 11
<i>T. pratense</i>	1, 2, 4, 5, 6, 8, 9, 11

The minimal number of biomorphs (two) has been recorded in the greater celandine *Chelidonium majus* L. and the maximum one (eight), in the red clover *Trifolium pratense* L. The variants can be classified according to different characters:

- (1) length of ontogeny;
- (2) monocentric, implicitly or explicitly polycentric patterns;
- (3) duration of the primary shoot preservation;
- (4) time, methods of growth and decomposition of the primary root;
- (5) formation of adventitious roots and type of root system (allorhize, allhomorhize, or homorhize);
- (6) formation of long or short rhizomes;
- (7) presence of single- or many-headed caudexes;

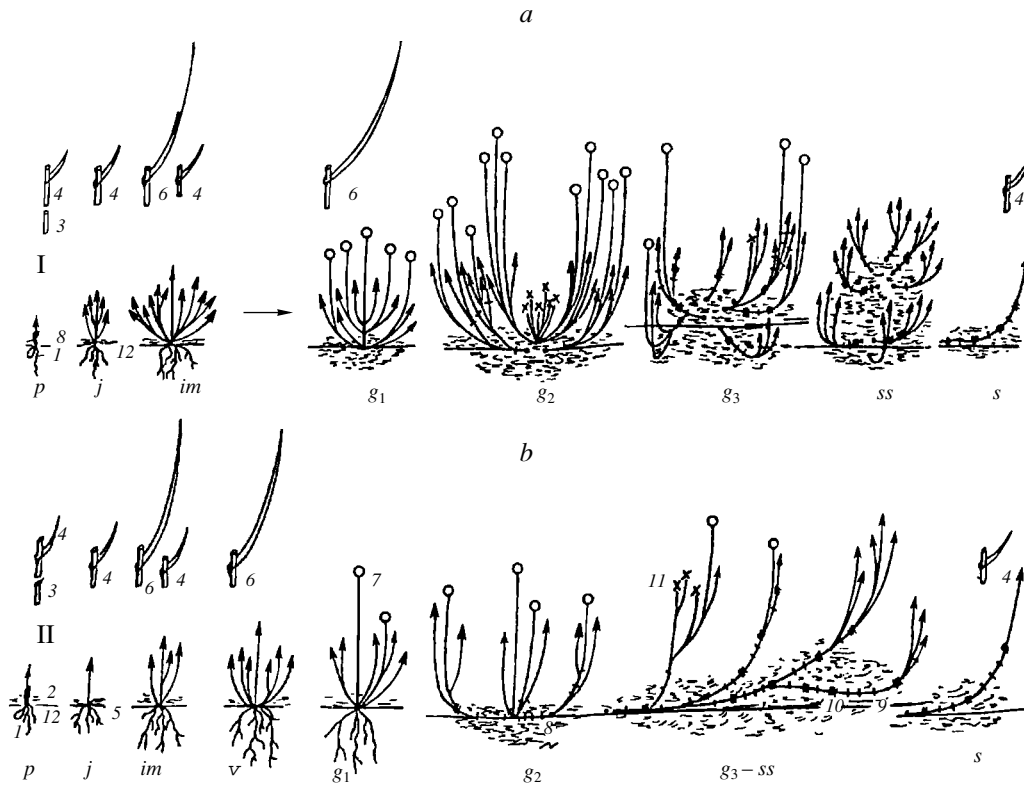
(8) transfer of the tillering zone or caudex and formation of upper-rosette, upper-semirosette, or middle-rosette shoots;

(9) specific functions of shoot and root systems: storing root, formation of the shoot system as a "cushion" or "baby's breath."

Let us consider the red clover biomorphs under different climatic and ecological conditions in more detail (Pokrovskaya, 1976). The tap root biomorphs with the principal rosette shoot occur more frequently in meadow phytocoenoses of the southern taiga: perennial (Fig. 1, 4), annual/biennial (Fig. 1, 2), annual (Fig. 1, 1), and, less frequently, caudex (Fig. 1, 8 and 9) plants with additional second order rosette shoots. A perennial tap root biomorph with a sympodial system of substitute rosette of the consecutive orders (Fig. 1, 6) appears in swamp meadows as well as a perennial raceme-root biomorph (Fig. 1, 11), which can be considered as an extreme adaptation to soil swamping. In the steppe zone, a perennial tap root biomorph (Fig. 1, 5) with an extended pregenerative period and tomentous petioles in plants of all ontogenetic states and a steppe tap root annual/biennial (Fig. 1, 2) with early dying principal rosette shoot and elimination of some stages of the generative period. In the taiga zone, the role of adventitious roots increases and the principal root system weakens, as compared to those in the steppe zone, thus suggesting a more active rearrangement of morphostructures and realization of another developmental program. Therefore, the movement of the red clover in the northern parts of its range is less restricted than in the south. Thus, different biomorphs of the red clover can act as indicators of climatic conditions.

Comparative-morphological studies of the underground organs of the ribwort *Plantago lanceolata* L. in continental meadows in the subzone of coniferous-broad-leaved forests (Zhukova and Osmanova, 1999) have shown that this species may be represented by monocentric, implicitly polycentric, and polycentric biomorphs. The described biomorphs occurred under different edaphic conditions and constitute an adaptation-morphological sequence: tap root (on sand substrate) → short-rhizomatous-tap-root (on cobble) → single-rosette short-rhizomatous raceme-root (on dern soil) → many-rosette short-rhizomatous (on loamy sand) → polycentric root-shoot (on slope upon plant burial). This suggests a high variability of the species and confirms the exceptionally important significance of morphological polyvariance which ensures realization of different variants of the complete ontogeny of genets and incomplete ontogeny of ramets of the shoot and root origin.

Hence, appearance of a wide range of biomorphs is related to diverse changes in ecological situations, such as shading, flooding, burial in sand, ground compaction, etc. Therefore, the earlier idea that every species has one life form is true only in a few cases of extreme



**Fig. 2.** Schematic diagram of age states of *Deschampsia flexuosa*: (a) monocentric or implicitly polycentric dense-cespitose biomorph; (b) polycentric stolon-dense-cespitose biomorph. (p) Plantlets, (j) juvenile, (im) immature, (v) virginil, (g<sub>1</sub>) young generative, (g<sub>2</sub>) mature generative, (g<sub>3</sub>) old generative, (ss) subgenile, (s) senile. (1) Germinal root, (2) rosette shoot, (3) coleoptile, (4) leaf of juvenile type, (5) older leaf, (6) leaf of adult type, (7) generative shoot, (8) stolon-semirosette shoot, (9) stolon-rosette shoot, (10) old zones of tillering, (11) dead shoot.

specialization (“cushions” or “babies’-breath” of tap root herbage).

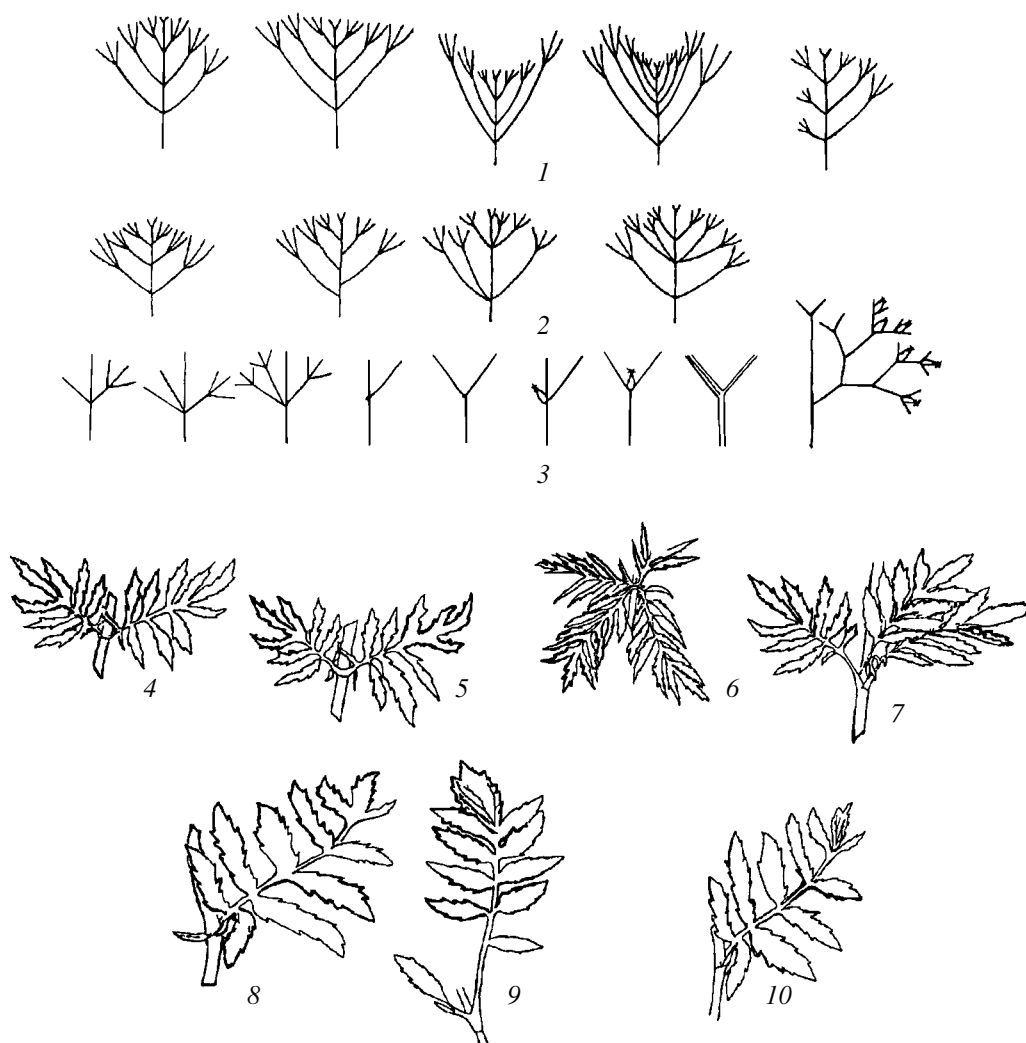
The diversity of life forms also determines that of ontogeny pathways, especially in the case of succession of biomorph types, when the monocentric biomorph becomes implicitly or explicitly polycentric. For example, two biomorphs were described for *Lerchenfeldia flexuosa* L. (Zhukova, 1979): monocentric or implicitly polycentric compact-cespitose on northern felled regions and polycentric stolon-compact-cespitose under the canopy of pine forests (Fig. 2). The plants of each biomorph realize their own ontogeny characterized both by duration and degree rejuvenation of ramets alternating in the population flow.

No less important consequences for the population life of plants are related to particulation at different stages of ontogeny. In cespitose grasses, cocksfoot *Dactylis glomerata* L., meadow fescue *Festuca pratensis* Huds. (Ermakova and Zhukova, 1985), and tufted hair-grass *Deschampsia caespitosa* P.B. (Zhukova, 1995), particulation proceeds in the old generative and subgenile states, as a rule, without rejuvenation of ramets. In some cases, however, reversions to the virginil state or, still more rarely, to the juvenile one are possible. Such a lability of the ramet behavior determines

the life span of coenopopulations and the rate of succession of its developmental stages.

Another aspect of morphological polyvariance is an increase in the number of shoots and dormant buds or laying down and development of adventitious buds on roots, rhizomes, or caudexes. This leads to the appearance of multi-rosette tap root, caudex, short-rhizomatous, and raceme-root plants and a number of transitional forms (Fig. 1) (Ontogeneticheskii atlas..., 1997, 2000), as well as to facultative root-shoot pattern: for example, in tap root polycarpous plants dandelion *Taraxacum officinale* L. (Ermakova, 1990), sickle medick *Medicago falcata* L. (Snagovskaya, 1965), and short rhizomatous herbage, including the ribwort (Zhukova and Osmanova, 1999).

The formation of stolons or creeping rhizomes in some grasses has a somewhat different morphological nature. For example, in *Lerchenfeldia flexuosa* and *Deschampsia caespitosa*, the first through third lower internodes of the rosette shoots could elongate and the latter could be transformed into upper-rosette or upper-semirosette shoots, thus leading to the appearance of stolon-tussock biomorph of the implicitly or explicitly polycentric type, which determines another method of assimilation and detention of the territory of a given



**Fig. 3.** Morphological polyvariance of *Valeriana officinalis* L.: (1) general appearance of inflorescence; (2) arrangement of first order paracladia; (3) arrangement of paracladia of the second and higher orders; (4) normal, opposite leaf arrangement; (5) opposite leaf arrangement, confluence of petioles; (6) shortened internode, whorl of four leaves; (7) whorl of three leaves; (8, 9) reduction of one leaf in opposite leaf arrangement; (10) alternate leaf arrangement.

coenopopulation. A similar picture could be observed in the greater plantain infected by powdery mildew, while its rosette shoots are transformed in middle- and upper-rosette shoots (Zhukova and Shestakova, 1995). Changes in the cyclicity of shoots have no less important consequences. After multiple hay-mowing on the Oka, Ugra, and Solotcha river meadows, the polycyclic shoots could be transformed into tri- or dicyclic shoots in the cocksfoot and fescue, while the duration of ontogeny was reduced.

At the same time, in loose-cespitose plants with mono- and dicyclic shoots, transition to polycyclicity is possible. This was recorded in sweet vernal grass *Anthoxanthum odoratum* L. (Zhukova, 1995) in Karelia and Irkutsk districts. The increase in shoot cyclicity leads, as a rule, to the longer life of ramets and slower development and turf degradation. This determines, in

turn, the time of particulation and formation of clones and their structure and dynamics.

Any deviations from the morphogenetic programs lead to morphological modifications and can be represented by changes in shape, edge, apex, base, and degree of segmentation of the leaf blade, pattern of leaf arrangement, increase in the number of elements of the generative organs, their confluence, elongation of the axial part of a flower or inflorescence, modification of the shape and color of flower elements, their reduction, etc. The scale of modifications is different: from variations in the leaf edge and coloration of petals to changes in the pattern of leaf arrangement, type of shoot, its cyclicity, transfer of the tillering zone, appearance of adventitious buds or roots, and metamorphosed vegetative organs.

If these modifications are preserved in the course of several ontogenetic stages, we deal with the morphological polyvariance of ontogeny. Those cases of morphological polyvariance that could change the population parameters are most significant for characterization of the populations. For example, the cases of appearance of whorl leaf arrangement on shoots of all-heal *Valeriana officinalis* L. instead of the typical opposite arrangement and reduction of inflorescence paraclydia are shown in Fig. 3. These modifications lead, in the first case, to an increased leaf surface and biomass and, in the second, to reduced seed productivity (Ilyushechkina, 1998).

Minute expressions of morphological variability in the vegetative and generative spheres occur in various herbaceous plants of most life forms. They were studied in detail for the all-heal, Greek valerian (Ilyushechkina, 1998), and trientale *Trientalis europaea* L. (Polyanskaya, 1999). The highest morphological biodiversity was recorded in the all-heal and trientale in the young generative state and the lowest at the old generative one. This may be related to the early death of individuals with morphological deviations, in agreement with the earlier data obtained for *Lysimachia vulgaris* L., *L. nummularia* L., and *Primula veris* L. (Primulaceae) (Zhukova, 1995). A positive correlation of phenotypic variability of the vegetative and generative in all ontogenetic groups of the generative period was shown for the all-heal.

The cumulative effect of different pollutants of the air basin and soil leads to a sharply increased (up to 80%) frequency of expressions of morphological polyvariance in the all-heal ontogeny. Unfavorable climatic conditions induce the same effect. At the same time, the morphological polyvariance of this species is fully absent or very rare in ecologically pure habitats or under optimal weather conditions. Hence, different expressions of ecological stress increase the level of intrapopulation diversity.

Further studies of morphological polyvariance will help solve some issues of the population biology of plants (Glotov and Zhukova, 1995):

(1) estimation of microgeographical distribution of biomorphs and morphogenetic disturbances within the territory occupied by a population, with special reference to heterogeneity of the habitat according to edaphic and phytocoenotic conditions, etc.;

(2) estimation of the degree and pattern of genetic determination of different biomorphs and morphogenetic disturbances; here, identification of the clones of vegetative-mobile species using protein and DNA markers can serve as a methodically important approach;

(3) estimation of the contribution of different biomorphs and morphogenetic disturbances in the next generation under different ecological conditions, i.e., determination of their role with respect to adaptation.

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## Genes Controlling Inflorescence Structure and Their Possible Role in Evolution

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**Abstract**—Structural features of the shoot system and inflorescence are the most important morphological features, on which plant systematics are based. The present review provides information on the genetic control of shoot morphogenesis in the model plant *Arabidopsis thaliana*. The results obtained made it possible to reveal a small group of genes responsible for the main taxonomic features of the shoot structure in this species. Cloning of these genes opened new avenues for directed search of homologous genes in other plants. Comparative analysis of the shoot structure and studies of the function and expression of these genes in various plants suggest that morphological evolution may be based on changes in the pattern of expression of single regulatory genes.

*Key words:* evolution of plant development, genetics of shoot morphogenesis.

Appearance of interspecific morphological differences is a fundamental problem of evolutionary biology. Any change in plant morphology is intimately associated with changes in developmental processes and, hence, with changes in the adaptive properties of development. What genetic changes underlie the morphological evolution of plants? An answer to this question would allow us to begin creating genetic models of morphological evolution. Advances in the developmental genetics of plants and molecular genetics during the recent 10–15 years cause a new trend in evolutionary biology to appear; this trend was called the evolutionary genetics of development. This discipline uses data from systematics, comparative plant morphology, developmental genetics, and molecular-phylogenetic analysis of the genes that control morphogenesis; it also studies genetic mechanisms that could underlie morphological plant evolution and allows estimation of the possible role of the described changes in the molecular structure of gene families in appearance of certain phenotypic changes. The conferences dealing with the problem of the evolution of development, an ever-increasing number of publications, and the publication of a new journal—“Evolution and Development”—suggest the intense development of studies in this field.

The question as to whether evolutionary changes are related to the gradual accumulation of mutational changes, each of them having a minimal phenotypic effect or are due to mutations of individual genes accompanied by significant morphological changes. The discussion of this question was until recently mostly theoretical and the opinions changed many times (Doebley and Luens, 1998). The main argument in favor of gradual transformations was the statement that the mutations of genes inducing significant pheno-

typic changes (“systemic mutations”) should decrease viability and fertility and be eliminated by stabilizing selection (Mayr, 1974).

However, recent studies have shown the existence of Goldschmidt’s productive “promising monsters” (Goldschmidt, 1940). Significant but not affecting viability phenotypic changes and, hence, cardinal changes in life strategy can be achieved at the expense of changes in single regulatory genes that govern many structural genes, the mutations affecting the pattern of expression being most promising. This concept theoretically substantiated most fully by Doebley and Lukens (1998) has been experimentally confirmed. Convincing examples of “happy monsters” supported by artificial selection were examples in the maize and wheat. Thus, the complex of most valuable features of the maize that distinguish it from its wild relatives is due to changes in single regulatory genes *TEOSINTE GLUME ARCHITECTURE1* (Dorweiler and Doebley, 1997) and *TEOSINTE-BRANCHED1* (Doebley *et al.*, 1997; Wang *et al.*, 1999). The dwarf wheat and maize cultivars involved in the “green revolution” have similar genetic genes in orthologous genes *Rht1* and *Rht2* (wheat) and *D8* and *D9* (maize) that code for transcription factors (Peng *et al.*, 1999). Apparently, the natural selection can also operate with regulatory genes in the course of evolution and select mutations that change the pattern of their expression. We will try to show the first successes achieved by evolutionary genetics of development in search of genes that could potentially underlie evolutionary transformations of the main morphological features of plants, specifically the features of shoot structure.

### GENES CONTROLLING THE SHOOT STRUCTURE

The cycle of the shoot system of herbaceous plants may be divided into several stages, and each species may pass only through some of them.

(1) Vegetative stage, which can be divided in two substages: (a) development of the rosette: internodes reduced, lateral meristems develop as the vegetative ones (characteristic for the flower plants); (b) development of the stem vegetative part (flower stalk in rosette plants): lateral meristems develop as the vegetative ones to form paracladia, long internodes (present in most plants).

(2) Stage of inflorescence formation: lateral meristems develop as floral ones, bracteoles may be reduced (present in all plants with lateral flowers).

(3) Stage of terminal flower formation: axils are inactive, leaves develop as flower organs, development of terminal meristem is arrested, gynoeceum is formed from its remnants.

The model genetic object wall cress *Arabidopsis thaliana* (L.) Heynh. is a rosette plant from the family Cruciferae and forms inflorescence as an open ebracteose raceme characteristic for most representatives of this family, i.e., *A. thaliana* plants pass through all stages of shoot development, except stage 3. Active studies on this plant revealed genes that control different stages of shoot development and determine the inflorescence structure and showed complex regulatory interactions between these genes. Let us consider the genetic control of individual stages of the shoot system development in *A. thaliana*.

*The gene responsible for rosette formation.* The dominant mutation *nana* (*na*) affects the formation of apical meristem of the flower stalk. The principal flower stalk does not develop in homozygotes for this mutation, thus leading to the initiation of buds in the axil of the rosette leaves and the formation of additional flower stalks, whose development was also defective. Their apical meristem forms, as a rule, one–two flowers and arrests proliferation, as a result of which the flowers are practically not elevated above the rosette surface (figure) and the mutant resembles *Jonopsidium acaule* plants, which form single flowers in axils of the rosette leaves. It was already shown that mutation *na* affects the sensitivity of plants to gibberellin (Ezhova *et al.*, 1997). Hence, the loss of sensitivity to this hormone, product of gene *na*, makes the plants incapable of flower stalk formation but does not deprive them of the capacity for flowering. The dominance of mutations *na* suggests that gene *NA* in wild plants at stage 1a of vegetative development suppresses the extension of internodes and flower stalk development, i.e., it is responsible for the formation of leaf rosette. At stage 1b, the negative effect of the product of gene *NA* is relieved under the influence of gibberellin, and the plant forms a flower stalk. Theoretically, the absence of the activity of this gene (in the case of recessive mutation) should

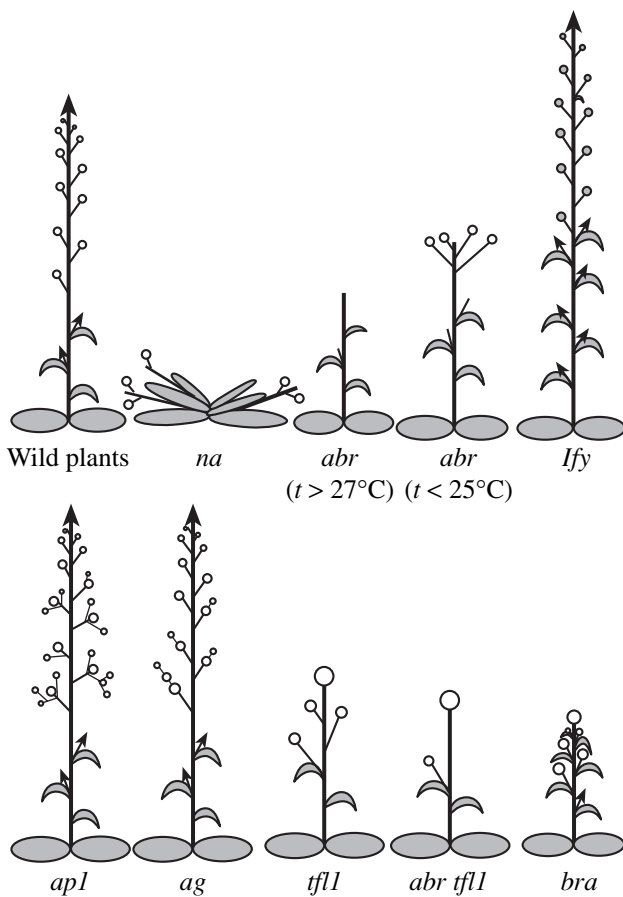
lead to the extension of all internodes and transformation of *A. thaliana* into a nonrosette plant.

*The genes marking the position of floral meristems.* The normal function of genes *PIN-FORMED* (*PIN*) and *ABRUPTUS/PINOID* (*ABR/PID*) is essential for flower stalk development at stage 2. The products of these genes are involved in the control of auxin polar transport in the flower stalk and marking of the position of lateral primordia. Gene *PIN* encodes the transporter protein that carries auxin from the basal part of cells in the apoplast (Galweiler *et al.*, 1998). Gene *PID/ABR* encodes a plant-specific serin-threonine protein kinase (Christensen *et al.*, 2000), which appears to be able to activate transporter proteins carrying auxin out of the cells, such as PIN-protein (Ezhova *et al.*, 2000). In mutants *pin* and *abr/pid*, the shoot development at stage 2 is not affected: a rosette and flower stalks with several cauline leaves are formed, the number of which is the same as in wild plants. The plants then pass to stage 2, but they cannot form floral meristems, and, as a result, the flower stalk resembles a pin (figure). Expressivity of mutation *abr* depends on the temperature (Ezhova *et al.*, 1999). At temperatures below 25°C, the plant forms an inflorescence, which resembles most often an umbrella due to a shortening of the upper internodes (figure) and disturbed proliferation of the apical meristem (it transforms into a pin-like structure). Apparently, protein kinase encoded by gene *ABR/PID* preserves partially its activity under these conditions; marking of the lateral primordia takes place but its patterns are disturbed.

Genetic analysis has shown that mutation *abr* is epistatic with respect to those in genes *LFY* and *API*, which are responsible for the formation of floral meristems: double mutants *abr lfy* and *abr ap1* have the same phenotype at 27°C as mutants *abr* (Ezhova *et al.*, 1997, 2000). Hence, the product of gene *ABR/PID* is necessary at earlier stages of the formation of floral meristem than those of genes *LFY* and *API*, which will be discussed below.

*Genes responsible for the formation of floral meristems.* The key role in the formation of floral meristems in *A. thaliana* is played by gene *LEAFY* (*LFY*), which is present in the genome in one copy and encoding the transcription activator that has no homologies with any known protein of animals and microorganisms (Weigel *et al.*, 1992). A weak expression of gene *LFY* is already observed at the vegetative stages, while active expression is recorded at the reproductive stage in the lateral regions of apical meristems, where the floral primordia are formed (Weigel *et al.*, 1992; Blázquez *et al.*, 1997; Hempel *et al.*, 1997). It has been proposed that achievement of the threshold level of gene *LFY* expression is a critical factor in switching the apical meristems from the formation of leaf primordia to the formation of floral meristems. Lateral shoots in the axils of cauline leaves are formed on the stem of mutants *lfy* instead of flowers (figure). Flowers with





Schematic diagram of the shoot structure in *Arabidopsis thaliana* mutants. Arrows show proliferating apical meristems of the principal and lateral shoots; in mutant *abr*, the apical meristems of the principal and lateral flower stalks is terminated by pin-like structures.

abnormal morphology may be formed in the upper layers of the inflorescence, with the organs located in a spiral, rather than as a whorl and reduced in number (Haughn and Sommerville, 1988; Schultz and Haughn, 1991; Weigel *et al.*, 1992).

Preservation of some flower features in mutants *lfy* is related to the activity of homeotic gene *API*, which encodes a transcription factor containing domain MADS (Mandel *et al.*, 1992) and, on one hand, regulates the development of flower organs of whorls 1 and 2 and, on the other, partially duplicates the function of gene *LFY* (Irish and Sussex, 1990; Bowman *et al.*, 1993). In mutants *apl* as well as mutants *lfy*, the forming flowers have some features of shoots: in axils of the first circle organs (in mutants *apl*, sepals are transformed into leaves), additional flowers are formed, which are able of forming I turn, the third order flowers (Irish and Sussex, 1990; Bowman *et al.*, 1993; Mandel and Yanofsky, 1995). As a result, the floral units are represented in the mutant, which can be classified as tiers (figure), rather than by individual flowers. Combined involvement of genes *LFY* and *API* in the

regulation of floral meristem formation follows from the phenotype of double mutants *apl lfy*: lateral shoots fully devoid of flower features are formed in them instead of in the flowers (Weigel *et al.*, 1992; Huala and Sussex, 1992; Schults and Haughn, 1993). Expression of gene *API* is positively regulated by a transcription protein encoded by gene *LFY*, which is connected with a regulatory element of gene *API* promoter (Parcy *et al.*, 1998; Wagner *et al.*, 1999; Sessions *et al.*, 2000).

The important role of genes *LFY* and *API* in the formation of floral primordia was also confirmed in experiments with transgenic *A. thaliana* plants, in which these genes were constitutively expressed under the control of an active promoter of gene *35S* of the cauliflower mosaic virus (Weigel and Nilsson, 1995; Mandel and Yanofsky, 1995; Liljegren *et al.*, 1999) or its own promoter (Blázquez *et al.*, 1997). The formation of plants in transgenic plants was observed much earlier and in those regions, where cauline leaves with axil buds were usually formed in wild plants. In addition, the formation of a terminal flower was also observed; i.e., not only lateral primordia, which are formed at the apical meristem sides, are transformed into floral meristems, but also the shoot apical meristem itself. Note that the ectopic expression of gene *35S::API* in mutants *lfy* did not lead to the normalization of flower morphology: they had multiple defects typical for mutants *lfy* (Mandel and Yanofsky, 1995). Hence, gene *API* affects the later stages of floral meristem formation more than gene *LFY*. On the other hand, transformation of shoot meristems into floral meristem in the plants with constitutive expression of gene *LFY* was partially suppressed by mutation *apl*, thus suggesting that phenotypic changes in *35S::LFY* plants are, at least partially, related to the activity of gene *API* (Weigel and Nilsson, 1995). Also note that both genes were capable of accelerating the flowering in other plants. For example, after transformation by genes *35S::LFY* or *35S::API*, the citrus plants already flowered during the second year, rather than within 6–20 years (Pena *et al.*, 2001).

The gene *CAULIFLOWER (CAL)* partially duplicates the function of gene *API*. Mutants *cal* have normal phenotype, but in double mutants *apl cal*, floral meristems are transformed into the inflorescence meristems and the inflorescence resembled that of cauliflower (Bowman *et al.*, 1993). Gene *CAL* encodes a MADS-containing transcription factor and has a high homology with gene *API* (Kempin *et al.*, 1995).

Gene *AG* also encodes the MADS-containing transcription factor (Yanofsky *et al.*, 1990) and is responsible for differentiation of organs of whorls 3 and 4 and for determination of floral meristem development (Bowman *et al.*, 1989, 1991). In mutants *ag*, stamens are replaced by petals and carpels by sepals; in addition, the flower becomes “many-storied” due to unlimited proliferation of floral meristems (figure). When mutants *ag* were grown during a short day, the floral

meristem was transformed into the shoot meristem and inflorescences with several flowers developed from the center of flowers (Okamoto *et al.*, 1996). The transgenic plants, in which gene *AG* (*s5S::AG*) was ectopically expressed, resembled plants with ectopic expression of genes *LFY* and *API*; i.e., they flowered earlier and formed terminal flowers on the main axis and on all lateral axes, thus suggesting the involvement of gene *AG* in the formation of floral meristems (Muzumoto and Ma, 1997). Gene *AG* and gene *API* are controlled by gene *LFY* (Busch *et al.*, 1999).

*Genes suppressing the terminal flower development.* In wild plants, transformation of the apical meristems of the main and lateral shoots into floral meristems is prevented by genes *TERMINAL FLOWER1* (*TFL1*) and *TERMINAL FLOWER2* (*TFL2*) (Shannon and Meekins-Wagner, 1991; Alvarez *et al.*, 1992; Ohshima *et al.*, 1997; Larsson *et al.*, 1998). Mutants *tfl1* and *tfl2* flower much earlier than wild plants and, soon after the induction of flowering, the shoot apical meristem transforms into the floral meristem. As a result, mutants *tfl1* and *tfl2* form a terminal flower, i.e., the open type inflorescence characteristic for *A. thaliana* transforms into closed-type inflorescence (figure). Formation of the terminal flower in mutants *tfl1* is related to formation in the absence of the normal product of gene *TFL1*, expression of genes *LFY* and *API* responsible for the formation of floral meristem, which is found not only in the peripheral regions of apical meristem, like in wild plants, but also in its central zone (Weigel *et al.*, 1992; Bowman *et al.*, 1993; Gustafson-Brown *et al.*, 1994; Bradley *et al.*, 1997). In double mutants *abr tfl1* at temperatures above 27°C, the terminal flower is also formed, and lateral flowers are absent due to disturbed activity of gene *ABR*. As a result, the mutant inflorescence consists of one terminal flower (figure).

The formation of terminal flowers in *A. thaliana* plants with ectopic expression of genes *LFY* and *API* suggests that these genes may negatively regulate gene *TFL1* activity (Weigel and Nilsson, 1995; Mandel and Yanofsky, 1995; Liljegren *et al.*, 1999; Ratcliffe *et al.*, 1999).

*Genes suppressing development of bracteoles.* In *A. thaliana*, like in most representatives of the family Cruciferae, the inflorescence is bracteose; i.e., the development of bracteoles is suppressed during transition of the plants to stage 2. It was believed until recently that gene *LFY* is responsible for the reduction of bracteoles, since in mutants *lfy*, the abnormal flowers are sometimes accompanied by bracteoles (Shultz and Haughn, 1991; Weigel *et al.*, 1992). The study of a new mutant suggests that the bracteole in *A. thaliana* is reduced mostly under the influence of the gene called *BRACTEA* (*BRA*) (Ezhova, 1999). In mutants *bra*, all flowers are laid down in the axils of bracteoles and a terminal flower is formed (figure). Thus, as result of the recessive mutation in one gene, the mutant inflores-

cence is transformed from open ebracteose into closed bracteose.

Analysis of the phenotype of double mutant *bra lfy* showed that it is more capable of forming bracteoles than both parental forms (Ezhova and Penin, 2001). This feature suggests that gene *LFY* may positively regulate gene *BRA* suppressing the development of bracteoles.

*Genes as potential participants of evolution of the shoot structure.* Thus, mutations were found in *A. thaliana* that led to changes in the taxonomic features of the shoot structure. Hence, the genes, in which mutations have arisen, are responsible for the development of a species-specific shoot. The activities of these genes determine the fate of meristems of the shoot and, hence, its structure. Identification of the genes controlling the taxonomic features by mutation analysis is the initial and central stage of studies in the field of evolutionary genetics of development. All of these genes are potential participants in the evolutionary transformations of the shoot structure. In order to confirm the possible involvement of genes in the evolution of morphological structures, it needs to be determined that orthologous genes exist in other plants and shown that the patterns of their expression are similar in species with a similar morphology of the taxonomic feature. It needs also to be ascertained that, in closely related species with a different morphology, the patterns of expression of orthologous genes are different. However, an allowance should be made that the changes in the pattern of expression of the gene in question need not to be related to those of its regulatory regions (*cis*-regulatory elements) but may be induced by those of upstream regulatory genes. For example, enhanced expression of gene *LFY*, early flowering, and terminal flower formation are observed in the case of ectopic expression of gene *ONSTANT* (*S35::CO*), which regulates the time of flowering and expression of gene *LFY* (Simon *et al.*, 1996). Thus, in order to clarify the true causes of morphological changes, it is necessary to study the genetics of morphogenesis. Experiments on transformation of closely related plant species by the gene in question are also a good means to examine the true relationship between the changes of morphological features and genetic changes of the gene in question.

The genes controlling the most important taxonomic features of the inflorescence structure have been identified not only in *A. thaliana* but also in other flowering plants. In a plant from the family Scrophulariaceae, snapdragon *Antirrhinum majus*, the formation of floral meristems is controlled by a gene orthologous to *LFY*, gene *FLORICAULA* (*FLO*), whose protein product has a high homology (70%) to protein *LFY* and which, like gene *LFY*, is present in the genome in one copy (Coen *et al.*, 1990). In *A. majus*, like *A. thaliana*, there is an open-type inflorescence, and gene *FLO* is expressed only at the apical meristem sides (in the forming floral meristem), rather than in its central zone.

In addition, gene *FLO* is expressed in the primordia of bracteoles (in *A. majus*, unlike *A. thaliana*, the flowers are located in the axils of bracteoles) (Coen *et al.*, 1990). After transition to the reproductive stage, mutants *flo* change the position of leaves from opposite to alternate, but form lateral shoots instead of flowers (Carpenter and Coen, 1990; Coen *et al.*, 1990). It has been shown that gene *FLO*, like gene *LFY*, regulates downstream regulatory genes responsible for subsequent differentiation of floral meristems (Hantke *et al.*, 1995; McSteen *et al.*, 1998).

Orthologs of genes *LFY/FLO*, genes *NFL1* and *NFL2* (Nicotiana *FLO/LFY*, were found in the tobacco *Nicotiana tabacum* (family Solanaceae), which has a closed-type inflorescence (Kelly *et al.*, 1995). Unlike genes *LFFY/FLO*, expression of genes *NFL1* and *NFL2* is also observed in the central zones of the inflorescence apical meristem, which leads to the terminal flower formation. An ortholog of genes *LFY/FLO*, gene *ALF*, was also found in another representative of the family Solanaceae, *Petunia hybrida*, which has a closed-type inflorescence. Mutation *alf* leads to the transformation of the terminal flower into a shoot (Souer *et al.*, 1998). Transformation of flowers into shoots is also characteristic of mutant *falsiflora* of tomato *Lycopersicon esculentum*. The protein encoded by gene *FALSIFLORA* has a 90% homology with proteins *NFL1* of tobacco and *ALF* of petunia and 80 and 70% homologies with proteins *FLO* and *FY*, respectively (Molinero-Rosales *et al.*, 1999). In the tomato, like in tobacco and petunia, the inflorescences are closed, and the expression of gene *FA* in wild plants is observed not only in floral meristems, but also in apical meristems.

Gene *UNIFOLIATA* (also called *PEAFLO*) of the pea *Pisum sativum* is responsible for the formation of floral meristems and is homologous to genes *LFY/FLO* but, unlike the latter, has one more function: it is involved in the control of morphogenesis of the pea compound leaf. This follows from the phenotype of mutant *uni* characterized by defective function of gene *UNIFOLIATA*: in the mutant, the formation of flowers is affected and shoot-like structures are formed instead. The compound leaf is converted into a simple one (Hofer *et al.*, 1997). An ortholog of *LFY/FLO* was found in *Eucalyptus globules*. This gene has the same pattern of expression as gene *LFY*. Transgenic *A. thaliana* plants carrying gene *ELF1* under the 35S promoter, like the 35S::*LFY* plants, form flowers instead of shoots (Southerton *et al.*, 1998).

In rice *Oryza sativa*, gene *RFL* was identified, which had the same number and position of introns as genes *LFY* and *FLO* and whose product had 44 and 48% homology, respectively, with those of the corresponding genes (Kyojuka *et al.*, 1998). Gene *RFL* was expressed in the apical regions of young whorls, rather than in floral meristems. It has been proposed that this gene is involved in the development of whorls and their branching, rather than in the formation of floral mer-

istems; i.e., it is not an ortholog of genes *LFY* and *FLO*. This also follows from the phenotype of *A. thaliana* plants that constitutively express gene *RFL* under the 35S promoter. Out of 100 transgenic plants, only two formed a terminal flower and all plants had developmental defects of cotyledons, leaves, petals, and stamens. The data obtained suggest that the genetic mechanisms underlying the floral meristem formation in grasses may differ from those in dicotyledonous plants and call for further genetic studies. Apparently, genes *FLO/LFY* and *RFL* originated from an ancestor gene, but their functions diverged in the course of evolution (Kyojuka *et al.*, 1998).

In pine *Pinus radiata*, two homologs of genes *LFY/FLO* were found: genes *NEEDLY* (*NLY*) and *PrFLL*<sup>1</sup> (Mouradov *et al.*, 1998). The products of these genes significantly differ from those of genes *LFY* and *LO*: they lack a proline-rich domain on N-end and an acidic region in the middle of the molecule, which are supposedly essential for the functioning of the protein as a transcription factor. A study of gene *NLY* has shown that it is expressed in the regions of development of the female and male strobiles and at the initial stages of their development. Transformation of the *A. thaliana* plants by gene *NLY* under the control of the 35S promoter (35S::*NLY*) led to an early flowering, metamorphosis of shoots into flowers, and terminal flower formation. Moreover, gene *NLY* under the control of a gene *LFY* promoter was capable of suppressing the phenotype of mutant *lfy-26*. These experiments suggest that gene *NLY* is a true ortholog of genes *LFY FLO*, despite the described structural differences from these latter. Homologs of genes *LFY/FLO* were also found in representatives of the order Gnetales, *Gnetum genmon* and *Welwitschia mirabilis*, but their functions were not analyzed (Frohlich and Meyerowitz, 1997). The presence of orthologs of genes *LFY/FLO* in gymno- and angiosperms suggests that these genes were involved in the origin of reproductive organs in the ancestor forms of seed plants.

Specific features of the expression of *LFY/FLO* orthologs in angiosperms with closed- and open-type inflorescences suggest also a possible involvement of these genes in the evolution of the inflorescence structure. Thus, in plants with open inflorescences, *A. thaliana* and *An. majus*, genes *LFY* and *FLO* were expressed in the lateral zones, rather in the central zone of the inflorescence apical meristem. In plants with closed inflorescences, like tobacco, tomato, *Imatiens balsamina*, and petunia, a significant level of expression of these genes was also recorded in the central zone of apical meristem, which led to the terminal

<sup>1</sup> The pine gene *PrFLL* appears to be involved in the formation of male reproductive structures, since it is expressed in young male strobiles rather than in female ones (Mellerowicz *et al.*, 1998); i.e., it is a paralog of gene *NLY*. This also follows from a rather low homology of the products of *NLY* and *PrFLL*: 61% (for comparison: the products of different genes, *LFY* and *FLO*, have a 70% homology).

flower formation (Kelly *et al.*, 1995; Pouteau *et al.*, 1997; Pnueli *et al.*, 1998; Souer *et al.*, 1998).

As was already noted, in the *A. thaliana* mutant *tfl1*, a terminal flower is formed on the inflorescence apex. In *An. majus*, there is also a mutant *centroradialis* (*cen*) with closed-type inflorescence (Bradley *et al.*, 1996). Orthologous genes *TFL1* and *CEN* encode highly homologous proteins (70% of homology) resembling phosphatidylethanol binding animal proteins, whose function is yet unclear (Bradley *et al.*, 1997; Ohsima *et al.*, 1997). Both genes are expressed in the central zone of apical meristems of the principal and lateral shoots and prevent the expression of the genes responsible for the floral meristem formation in this zone (Shannon and Meeks-Wagner, 1993; Gustafson-Brown *et al.*, 1994; Ratcliffe *et al.*, 1998, 1999). Orthologs of genes *TLF1* and *CEN* were also isolated in the tobacco. These genes, *CET2* and *CET4*, have a 97% homology of coding gene region due, apparently, to the duplication of one ancestor gene in allotetraploid tobacco. The proteins encoded by these genes have a 83% homology with the product of gene *CEN* (Amaya *et al.*, 1999). The genes are expressed in apical meristems of the lateral shoots at the initial developmental stages, rather than in the apical meristem of the principal shoot. When the lateral shoots begin to flower, the expression of these genes in the shoot apical meristem ceases. The absence of the expression of genes *CET2* and *CET4* in the apical meristem of the principal shoot and related expression of genes *NFL1* and *NFL2* in this region (Kelly *et al.*, 1995) lead to terminal flower formation and inflorescence closure.

Thus, comparative analysis of the inflorescence structure in various plants and the pattern of expression of orthologs of genes *LFY/FLO*, which induce the formation of apical meristems, and *TFL1/CEN*, which maintain an undifferentiated state of apical meristems, suggests that the site of expression of these genes determines the formation of open or closed type inflorescences. Deviations from these patterns have also been described. Thus, a homolog of the tomato genes *CEN* and *TFL1*, gene *SELF-PRUNING* (*SP*) is expressed in all regions of the apical meristem (Pnueli *et al.*, 1998), which, however, does not prevent the expression in it of an ortholog of genes *LFY/FFLO* and the formation of the closed inflorescence (Molinero-Rosales *et al.*, 1999).

In *Jonopsidium acaule*, unlike most species of the Cruciferae, the flower stalk is not formed and individual flowers develop directly from axils of rosette leaves. Other *Jonopsidium* species, as well as species of the closely related genus *Cocchlearia*, form flower stalks, and, hence, the loss of flower stalk in *J. acaule* may result from rather simple genetic changes of ancestor forms with flower stalks (Shu *et al.*, 2000). Genes *vcLFY1* and *vcLFY2* were found in *J. acaule*, which showed an approximately 90% similarity of nucleotide sequences of exons with the *A. thaliana* gene *LFY*.

Active expression of these genes was observed not only in the floral meristems, but also in the bracteoles, like in the snapdragon, and shoot apical meristem. A similar pattern of expression, early flowering, formation of flowers in axils of the rosette leaves, and development of bracteoles were also observed in *A. thaliana* and petunia, which constitutively express gene *LFY* under the 35S promoter (Weigel and Nilsson, 1995; Souer *et al.*, 1998). This made it possible to propose that early flowering and formation of flowers from axils of the rosette leaves in *J. acaule* is also due to an enhanced expression of genes *vcFLY* (Shu *et al.*, 2000). However, it remains unclear why the flower stalk and terminal flower are not formed in *J. acaule* (they are present in transgenic plants). It cannot be excluded that the absence of a flower stalk is related to the changed expression of other genes, whose action is directed to suppression of the flower stalk development, such as the ortholog of gene *NA*, which was mentioned above.

Thus, the results of these studies suggest the existence of basal genes that control taxonomic features of the shoot system and whose orthologs are present in various plants. Regulatory genes have also been identified that control taxonomic features of the flower structure (MADS-containing genes that control differentiation of the flower organs and genes controlling the actinomorphic/zygomorphic feature) and leaf structure (homeobox-containing *KNOTTED*-like genes) (for reviews see Coen and Nugent, 1994; Baum, 1998; Theissen, 2000). Experimental studies on mutants and transgenic plants as well as studies of the pattern of expression of these regulatory genes in the species with different structure of the shoot, flower, or leaf confirm the hypothesis that changes in the pattern of expression of individual regulatory genes is a type of genetic variation, which may provide important materials for natural selection and underlie evolutionary transformations of morphological structures.

Genes controlling taxonomic features may be used in molecular-phylogenetic studies. Chloroplast genes, such as *rbsL*, and nuclear rRNA genes were previously used in most studies on molecular systematics. These genes are present in many copies and encode the most important structural proteins. The changes in nucleotide sequences of these genes, described during molecular-phylogenetic analysis, are, as a rule, neutral and occur at random in the area of accessible variants that do not affect the function. Random character of such neutral changes, which may lead to a semblance of relatedness, appears to be the main cause of controversies between the data of molecular systematics and phylogenetic analysis based on comparative morphology (Doyle, 1998; Frohlich, 1999).

Most genes controlling morphological taxonomic features are regulatory. Duplications of these genes and subsequent structural changes may lead to changes in their function due to altered specificity of binding to DNA or succession of protein partner (Frohlich, 1999).

If we take into account that the changes in these genes are related to morphological changes, it becomes evident that they should be subject to intense directed selection. Nonrandom structural changes of the genes that control taxonomic features and are identified by molecular-phylogenetic analysis, as well as their intimate relationship with morphological changes make these genes a very valuable tool in molecular-phylogenetic studies and allow estimation of the role of changes in the molecular structure in morphological evolution (Kramer *et al.*, 1998; Winter *et al.*, 1999; Theissen *et al.*, 2000). Identification of genes controlling the most important taxonomic features of the plant morphological structure, studies of molecular evolution of these genes, and analysis of the pattern of their expression in plants from different taxa are new approaches in evolutionary biology that allow us to look forward to the development of a new genetic model for the morphological evolution of plants.

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## Morphological Diversification of Fish as a Consequence of the Divergence of Ontogenetic Trajectories

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**Abstract**—The possibilities of the investigation of ontogenetic changes in the morphological features of fish using multidimensional “ontogenetic channels,” within which individual ontogenetic trajectories run, are demonstrated using African barbs (*Barbus intermedius* complex) as an example. The position of ontogenetic channels allows one to judge how the differences of adult individuals in morphological characters arise during development and how their morphological diversity is formed.

*Key words:* ontogenetic channels, *Barbus intermedius*, principal components analysis, skull proportions, populations, sympatric speciation.

### THE INVESTIGATION

When speaking about the morphological diversity of representatives of a certain group (taxon or population) of animals, including fish, one usually keeps in mind the diversity of adult individuals that are characterized by the sum of differences between these individuals in phenotypic characters. The main expression of evolution consists in diversification—an increase in morphological diversity of descendants of a common ancestor—which leads, in the long run, to the formation of morphologically different species and taxa of higher ranks.

When describing the morphological difference between adult individuals that represent groups originating from a common ancestor, it can be proposed that the path of formation of a given character in ontogenesis has changed.

The ratio of ontogenetic and phylogenetic changes was studied repeatedly (Gould, 1977; Alberch *et al.*, 1979; Heterochrony..., 1988), and the urgency of studies in this field raises no doubts. The problem concerns how such studies should be conducted with natural populations. We will try to consider this problem.

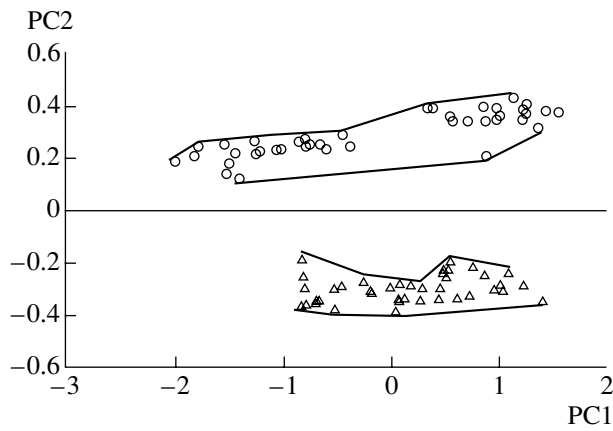
When comparing adult individuals that represent different species and intraspecific groups of fish, the most impressive differences are observed in morphological proportions. In order to understand how these differences arise, it is necessary to follow the formation of proportions in the ontogenesis of individuals under comparison. In the ideal variant, we should operate with lines describing changes in the position of an individual in a character space during ontogenesis, i.e., “ontogenetic trajectories” (Alberch *et al.*, 1979), rather than with points that represent individuals in such a

space. However, it is clear that, when we deal with natural populations, it is practically impossible to obtain the data necessary for plotting such lines. The shape of an ontogenetic trajectory still needs to be assessed from the description compiled on the basis of “statical” data. This description represents a line plotted from estimates of the character states in individuals of different ages.

When it is not possible to determine the age of every individual with sufficient accuracy, one is forced to limit oneself to considering the proportions as a function of size and reduce the task to analysis of allometry in a broad sense of the term, not implying a certain form of mathematical expression of the studied dependence of the form parameter from the size of individual (Gould, 1966). The line of individual ontogenetic allometry is identified with the individual ontogenetic trajectory although, strictly speaking, these notions are not equivalent, since individuals of the same size may have different ages, so that, for example, the coinciding ontogenetic trajectories may correspond to noncoinciding lines of ontogenetic allometry.

The problem of relations of the trajectories described by the results of tracking certain individuals and those plotted from estimates of the character states for different individuals has been considered in detail (Richards and Kavanagh, 1945; Cock, 1966; Mina and Klevezal, 1976). The main conclusion is that the more real the ontogenetic trajectories differ from each other, the worse the line plotted from statical data describe these trajectories. The line plotted from statical data may be positioned differently with reference to the real ontogenetic trajectories depending on the pattern of differences between these trajectories (Mina and Klevezal, 1976). If sufficient statical data are available,





**Fig. 1.** Ontogenetic channels of barbs *Barbus intermedius* of the generalized form from the Gumara River (○) and *Varicorhinus beso* from Lake Tana (△). Here and in Figs. 2–5: (PC1 and PC2) the first and second principal components, respectively; each point represents an individual.

the area of the character space may be determined, where the real ontogenetic trajectories of individuals from the sample in question are located. We call this area the “ontogenetic channel” (Mina *et al.*, 1996a).

Loadings of 14 characters on the second principal component (vector length equals 1)

Character	Scatter-diagrams				
	Fig. 1	Fig. 2	Fig. 3	Fig. 4	Fig. 5
BL	0.335	0.008	0.165	0.056	0.025
B1	-0.350	0.288	-0.451	0.322	-0.490
B2	-0.017	-0.095	-0.138	0.180	0.011
B3	-0.083	0.083	-0.270	0.222	-0.167
B4	-0.118	0.344	-0.326	0.208	-0.652
HS1	-0.140	0.530	-0.201	0.160	0.113
HS2	-0.128	0.183	-0.093	0.131	-0.054
Hm	-0.180	-0.077	-0.133	0.264	0.123
Pmx	-0.239	-0.452	0.364	-0.476	0.276
Pop	0.257	-0.043	0.131	-0.087	0.037
Op	0.000	-0.039	-0.035	0.108	0.105
Iop	0.547	-0.043	0.311	-0.407	0.105
Mx	-0.171	-0.465	0.241	-0.282	0.191
De	0.474	-0.208	0.446	-0.406	0.373

Note: (BL) basal skull length, (B1, B2, and B3) distances between the external edges of *frontalia*, *pterotica*, and *sphenotica*, respectively; (B4) skull width at the level of *frontalia* and *pterotica* junction; (HS1 and HS2) skull depths at the level of bend and of the posterior edge of *paraspheoideum*, respectively; (Hm, Pmx, Pop, Op, Iop, Mx, and De) measurements characterizing the sizes of *hyomandibulare prae-maxillare*, *praeoperculum*, *interoperculum*, *maxillare*, and *dentale*, respectively.

## ONTOGENETIC CHANNELS: AN EXAMPLE OF USE

Identification of ontogenetic channels is of special interest in those cases when it is possible to compare samples from populations, where phyletic relationships are known and adult individuals from different populations or intrapopulation groups substantially differ in morphological characters. Barbs of the Northeast Africa *Barbus intermedius* complex sensu Banister (Banister, 1973) can serve as an example, and we will consider this example on the materials collected by the Joint Ethiopian-Russian Biological Expedition in 1992–1999.

The *Barbus intermedius* complex is represented in rivers and lakes in Ethiopia in many forms, among which a “generalized” form can be identified that has an inferior mouth, a head rounded in front, and a short snout and lacks nuchal hump; i.e., in these individuals, dorsal contours gradually transform to head contours. The khramulya *Varicorhinus beso* Rüppell, 1836, occurs in many bodies of water together with barbs. This species is phylogenetically close to barbs but differs from them distinctly in some morphological features, above all, their head structure.

Figure 1 demonstrates the ontogenetic channels of khramulya from Lake Tana and a generalized form of barbs from a tributary of this lake, the Gumara River. In this case, like in subsequent examples, the results of principal components analysis of 14 skull measurements were used. The scheme of measurements was given elsewhere (Mina *et al.*, 1996a). Logarithms of the absolute values of measurements were analyzed, which were standardized by the standard deviation. Following the widely accepted interpretation (Tissot, 1988), the first principal component (PC1) was considered a characteristic of the size of an individual or, to be more precise, its skull, while the subsequent components (PC2, PC3, etc.) as characteristics of the skull shape (proportions). The characters that determine the differences in the position of ontogenetic channels along the axis PC2 could be inferred from the eigenvector loadings (table).

The ontogenetic channels of the barb from the Gumara River and khramulya were separated over the entire interval of sizes considered. Of the studied fish, the smallest were two years old, while the age of the largest, sexually mature individuals was no less than five years. Thus, it can be seen that the differences in proportions of the barb and khramulya skulls are determined during the first year of life and are preserved later. The main differences are that the skull is wider with reference to length in the khramulya than in the barb, while the interoperculum (Iop) and dentale (De) are shorter (table).

Figure 2 shows the ontogenetic channels of the barbs from populations of different river basins distinctly isolated from each other. Both populations represent the generalized form. The samples were used from the Gumara River (Blue Nile basin) and Gelgel

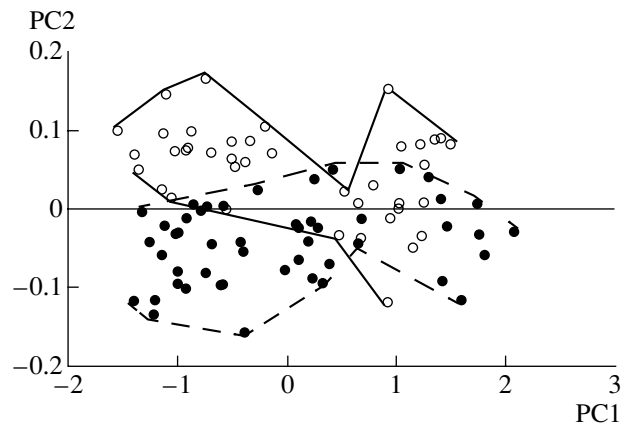
Gibe River (Omo basin). The ontogenetic channels overlap significantly. Note, however, that at  $PC1 < -0.7$  (age 2–3 years), no overlapping was observed, and the differences consist in that in barbs the skull is deeper (HS1), while the praemaxillare (Pmx) and maxillare (Mx) are shorter.

The recognition of generalized form does not imply the absence of morphological differences between individuals and groups of individuals that represent this form. The generalized form is identified, because these differences are much less than those observed between all its representatives and representatives of the groups that refer to other forms.

In addition to the generalized form, the *B. intermedius* complex also contains forms that differ from it and from each other in body and head proportions. These forms do not occur in all water bodies in Ethiopia where the generalized form is found, but always together with this form. In some water bodies, various forms constitute so-called flocks—monophyletic groups. A flock in aforementioned Lake Tana, the largest lake of Ethiopia (ca. 3500 km<sup>2</sup>) is well-known. Fourteen forms (morphotypes) of Tana barbs have now been identified (Nagelkerke *et al.*, 1994, 1995). One can say with a fair degree of confidence that all of them originated from barbs of the generalized form that are similar to those represented in our materials by the sample from the Gumara River (Dgebuadze *et al.*, 1999). These forms differ in body depth, snout length, mouth position, presence or absence of nuchal hump, etc. It is difficult to evaluate quantitatively these differences when taken together, but, according to a visual estimate, they are comparable to the differences between representatives of different genera of the European cyprinids. However, the forms differ little in meristic characters (Banister, 1973; Mina *et al.*, 1996b) and only one of them (morphotype “acute”) differs significantly from other forms in the allelic frequencies at allozyme loci (Berrebi and Valiushok, 1998).

Most individuals that are more than 25 cm in length (standard length) can be referred to one of the forms on the basis of description and illustrations provided by Nagelkerke *et al.* (1994, 1995). Individuals of the form with an acute snout and the form with large eyes can also be identified at a smaller standard length. But the differences in the presence or absence of nuchal hump and in the position of mouth (inferior/terminal or superior/terminal) are distinctly expressed only in large fish. Until these differences are determined, individuals with characteristic features of different forms are externally similar to each other and those of the generalized form.

However, large individuals of different forms differ not only in their external features, but also in skull proportions, and the origin of these differences may be followed through ontogenetic channels from the results of skull measurements. The ontogenetic channel of the “acute” form is separated from those of other forms during the first year of life. Figure 3 shows onto-



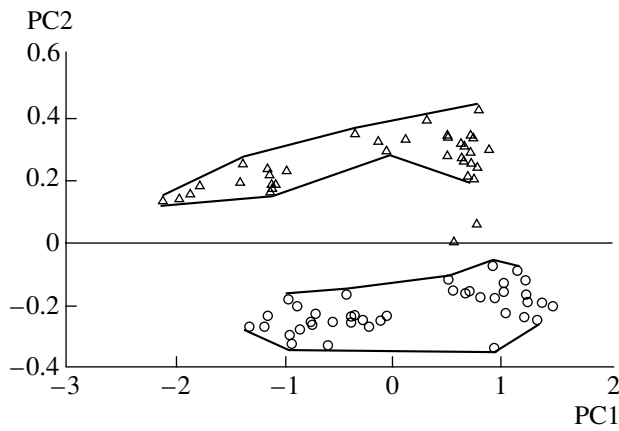
**Fig. 2.** Ontogenetic channels of barbs *Barbus intermedius* of the generalized form from the Gumara (○) and Gelgel Gibe rivers (●).

genetic channels of the “acute” and the generalized form represented by a sample from the Gumara River. The “acute” sample includes individuals, whose length does not exceed that of the Gumara individuals. The forms compared differ mostly in skull proportions (the skull is narrower and less deep in the “acute” form) and in the size of the bones that form jaws (Pmx, Mx, and De) and lop (in the “acute” form, these bones are longer) (table).

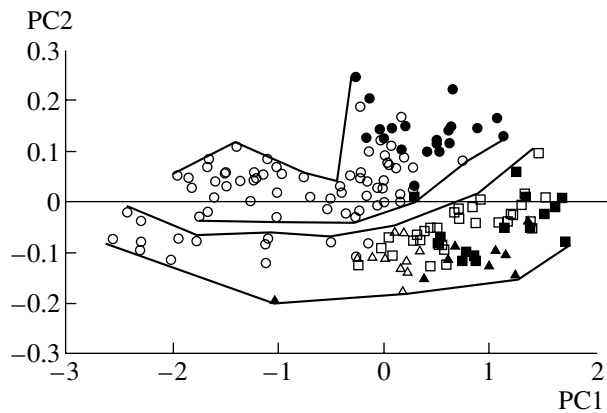
Analysis of a sample including individuals of the generalized form from the Gumara River and Lake Tana allows us to distinguish two ontogenetic channels (Fig. 4). The samples included those considered by Nagelkerke *et al.* (1994) as a separate morphotype “carplike” and individuals of four other forms, such as humpbacked with a light coloration and subsuperior mouth (morphotype “white hunch”), a deep-bodied form similar to the previous one but without a distinct hump (“troutlike”), a form with a low cylindrical body and terminal mouth (“bigmouth mini-eye”), and a form with unusually thick and long barbels (“barbel”).

These channels are already separated in the beginning of the studied period of ontogenesis (second year of life). One of them contains individuals that preserve morphological features of the generalized form throughout this period, morphotype “carplike” in its terminal part. Another channel comprises the barbs that have external features of the generalized form for a long time (until the fifth–sixth year of life) and then acquire features of any of the four morphotypes: “white hunch,” “troutlike,” “bigmouth mini-eye,” or “barbel.” Taken together, these morphotypes are characterized by larger sized Pmx, Mx, De, and lop than in the generalized form, and their skulls are narrower and less deep (table).

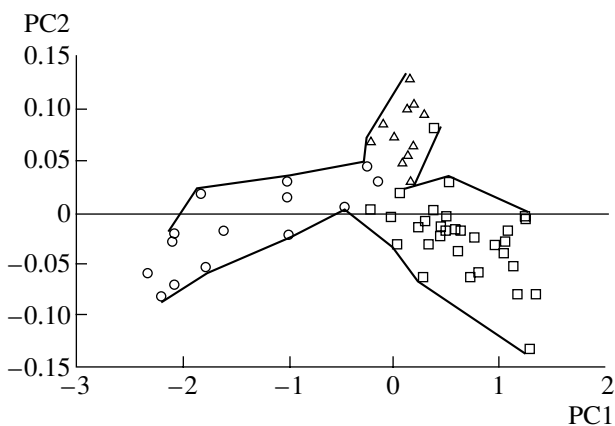
When including these morphotypes in pairs in the principal components analysis together with individuals of the generalized form located in the same channel (Fig. 4), it can be seen that each morphotype has its own



**Fig. 3.** Ontogenetic channels of barbs *Barbus intermedius* of the generalized form from the Gumara River (○) and the “acute” form from Lake Tana (△).



**Fig. 4.** Ontogenetic channels of barbs *Barbus intermedius* of the generalized form from the Gumara River and Lake Tana (○) and forms (morphotypes) “carplike” (●), “white hunch” (□), “barbel” (■), “bigmouth mini-eye” (▲), and “troutlike” (△).



**Fig. 5.** Ontogenetic channels of barbs *Barbus intermedius* located in the same ontogenetic channels in Figure 4: generalized form (○) and morphotypes “white hunch” (□) and “troutlike” (△).

ontogenetic channel displaced with reference to the channel of any other morphotype due to the differences in specific combination of characters' states. For example, divergence of “troutlike” and “white hunch” channels (Fig. 5) is mostly due to the differences in the ratios of measurements characterizing the skull width (table). (The presence of one “white hunch” individual in the “troutlike” channel in Fig. 5 is, in all likelihood, due to the erroneous visual identification of its morphotype.)

All segments of ontogenetic channels, where the morphotypes are recognized by external features, are interlocked with a segment, where only individuals of the generalized form caught in the lake are represented. Sharp changes of morphological proportions in the late ontogenesis were noted in fish of different taxa, specifically in salmonids (Savvaitova, 1989) and cyprinids (Dgebuadze, 1995). In all these cases, however, such changes were coupled with an increased growth rate unlike in the Tana barbs (Mina *et al.*, 1996b). It may well be that different developmental programs are realized in this case, but it is not known what programs these are and how they operate.

When studying only large, mostly adult, Tana barbs, all morphologically different groups (forms or morphotypes) are considered equivalent units. Some authors consider all of these groups independent species (Rüppell, 1836; Boulenger, 1911; Bini, 1940; Nagelkerke and Sibbing, 1997), while others refer to them as the same species, *B. intermedius* Rüppell, 1836 (Banister, 1973). In the first case, it is virtually maintained that the forms represent reproductively isolated populations, while in the second case it is assumed that they belong to the same population.

Having identified ontogenetic channels, we can propose another interpretation of the observed situation (Mina *et al.*, 1996b). According to this interpretation, sympatric speciation takes place in the Tana barbs, which is described by Kondrashov's model (Kondrashov, 1986; Kondrashov and Mina, 1986; Kondrashov and Kondrashov, 1999). And, ontogenetic channels of different forms are found at different stages of divergence determined by the disruptive selection. This selection is related to utilization of different food resources by different forms (Sibbing *et al.*, 1998). Among the considered forms, “white hunch,” “barbel,” “bigmouth mini-eye,” and “troutlike,” are at an earlier stage of divergence, while “acute” is at a later stage.

This approach implies that individuals of the same form mate predominantly with each other, but there is no full reproductive isolation in any of the considered cases, as follows from a significant number of individuals with intermediate skull proportions, even between the most separated “acute” form and the generalized one (Fig. 3). In this situation, it is impossible to distinguish species that fully correspond to the biological species concept, i.e., that are isolated gene pools. It is quite likely, however, that, under certain conditions, some form will acquire the status of biological species

and that some sympatric forms of barbids found outside the Lake Tana basin are different species (Mina *et al.*, 1998).

In summary, it is evident that, although ontogenetic channels just roughly describe the developmental pathways, their plotting and analysis make it possible to consider in more detail morphological diversification of animals, such as fish, in nature and make the tasks of this consideration more specific.

Investigation of the young of the year, starting from the time of formation of the skull as an integral morphological structure, may provide a better understanding of the mutual position and divergence of ontogenetic channels.

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## Retrospective Estimate of the Individual History of Life in Population Studies on Mammals

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**Abstract**—Analysis of the recording structures in mammals, such as dentine, cementum, or bone tissue, makes it possible to estimate the parameters of individual history of life: age at the moment of death, seasons of death and birth, growth rate, age of sexual maturation, etc. Using the reindeer (*Rangifer tarandus*) as an example, it has been shown what specific life features essential for population studies may be estimated when only mandibles with teeth are available.

*Key words*: recording structures, reindeer.

### AIMS OF STUDY

All theoretical studies, including mathematical modeling of the processes taking place in populations, are based on data on the individual history of life: life span, growth pattern, age of sexual maturation, fertility, etc. The solution to problems like “aging” in natural populations of mammals, when aging is defined as an increase death probability with the age (Promislow, 1991), requires reliable facts (Gaillard *et al.*, 1994). Such data can now be obtained using the recording structures of mammals, dentine, cementum, or bone tissue, which record, during their growth, changes in the physiological state of an individual in the form of layers of different orders (Mina and Klevezal, 1970; Klevezal, 1998).

The following parameters of the individual history of life can be estimated from analysis of the recording structures (Klevezal and Kleinenberg, 1967; Klevezal, 1988, 1996, Klevezal and Sokolov, 1997, 1998; Trunova and Klevezal, 1999; Trunova *et al.*, 1999):

—age at death (in almost all mammals—based on the number of annual layer in dentine, cementum, or bone);

—season of death (in most mammals—based on the forming part of annual layer at death);

—season of birth in mammals that reproduce throughout the entire year or identification of early or late born in mammals that reproduce seasonally—based on the position of the first annual layer of cementum with reference to the root dentine part;

—growth rate and age of sexual maturation (in long-living mammals—based on the width of annual layers in bone tissue or dentine and the ratio of the number of layers in the secondary dentine to cementum);

—number of acts of reproduction (in some long-living mammals—based on marks of reproduction in annual layers);

—phase of sexual cycle at the moment of death (in some rodents—based on the character of annual layers in the incisor dentine);

—specific features of feeding (in some long-living mammals—based on the pattern of layers in dentine);

—specific features of hibernation and time of awakening (in hibernating rodents—based on the “hibernation zone” and the annual layer in the incisor dentine).

Below, using the reindeer *Rangifer tarandus* as an example, I will show what specific features of the individual history of life essential for population studies may be estimated when only mandibles with teeth are available.

### METHODS

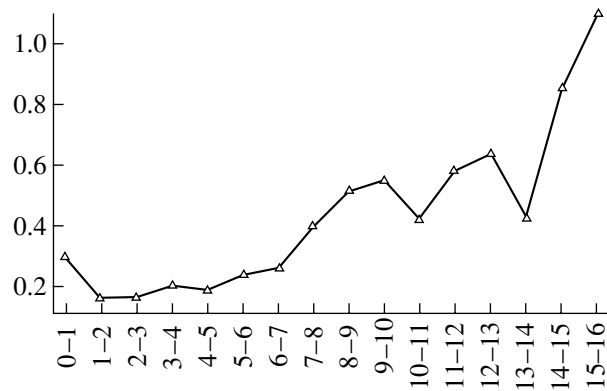
The mandibles and diastemas were measured (distance from the last incisor to the first cheek tooth), M1 was removed from the alveole and decalcified, and longitudinal sections of roots passing through the pulp channel and cross sections of the lower crown part were made. The sections were stained with hematoxylin and embedded in glycerol. The tooth cementum and dentine were examined under dissecting and light microscopes (Klevezal, 1988). When studying the preparations, the age of an individual at the moment of death was determined according to the number of annual layers in dentine. The age of sexual maturation was determined based on a comparison of the number of layers in the secondary dentine and cementum. And, the season of death was determined according to the part of dentine and cementum annual layer forming at the moment of

death. In addition, animals born at the end of the breeding season were identified according to the position of the first winter increment line in the cementum with reference to the root dentine part (on longitudinal sections).

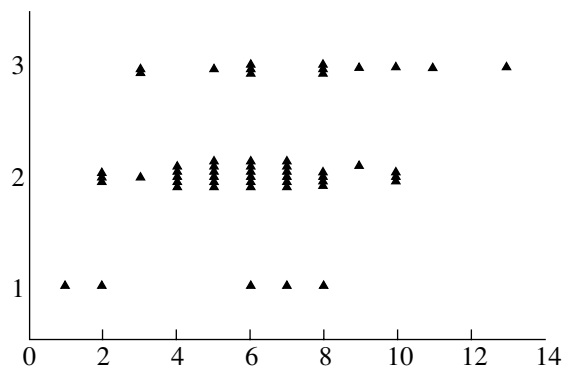
### ESTIMATED PARAMETERS

*Life span and dynamics of death.* In cases when mandibles were taken from animals that had died in nature, age determination from annual layers enables us to plot a demographic table and determine age-related changes in the probability of death and maximum life span. Reimers (1983) determined the age of reindeer, whose mandibles were collected on the island of Spitzbergen between 1973 and 1978. There are almost no reindeer predators on Spitzbergen, and these animals have not been hunted there since 1925. The curve of age-related changes in the probability of death in this population of reindeer (Fig. 1) is typical for long-living mammals (Hutchinson, 1978; Heligman and Pollard, 1980): a high mortality rate for young animals, its decrease when the animals reach the age of two, and subsequent exponential increase. (Note that the mortality of the young animals for this year is understated in this case, since their mandibles are poorly preserved—Reimers, 1983). The lowest death probability was noted at ages ranging from two to three years and the maximum life span was 15 years.

The data obtained is based on large amount of factual material and may serve as a foundation for comparing mortality rates in other populations of the same species. Cases may be of particular interest, in which the increase in the death rate is found at an age when the death probability is normally low, especially if the season of death can be determined. Thus, the curve of mortality plotted on the basis of analysis of the reindeer mandibles collected in the Novaya Zemlya tundra (Klevezal and Sokolov, 1999) showed that an increased proportion of deer died between the ages of one to three years compared to Spitzbergen reindeer: 18% ( $n = 107$ ) versus 10% ( $n = 388$ ). When the season of death for Novaya Zemlya reindeer was determined, it turned out that 71% of all animals died during the unfavorable low-diet winter-spring period, which is characteristic of all reindeer. Note that the increase in the mortality rate between the ages of one and three years was due to the death of deer during the favorable summer-autumn period. The two-year-old and three-year-old group was the only group, in which the mortality rate during the summer-autumn period was higher than during the winter-spring period (Klevezal and Sokolov, 1999). The question now arises about the cause of the unusually high mortality rate during the summer-autumn period. In the case of Novaya Zemlya reindeer, their irradiation as a result of nuclear tests was considered as a possible cause (Klevezal *et al.*, 1997; Klevezal and Sokolov, 1999).



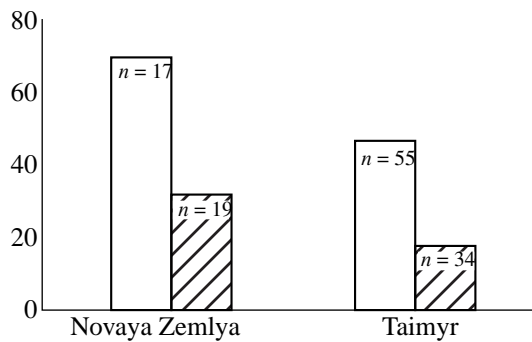
**Fig. 1.** Changes in death probability (ordinate) in reindeer as a function of age (abscissa, years) (total of males and females,  $n = 388$ , data of Reimers, 1983).



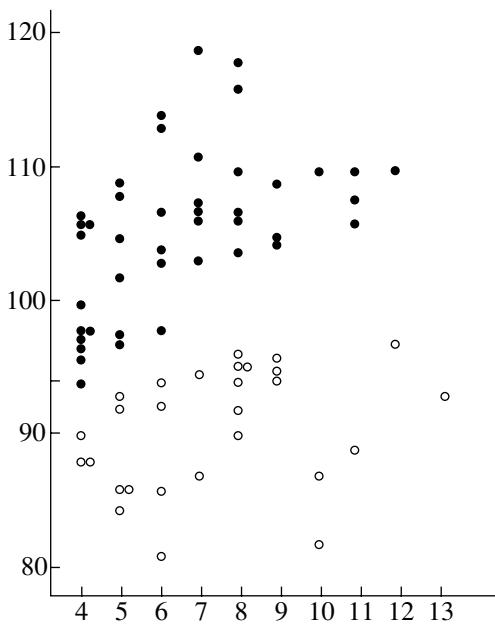
**Fig. 2.** Relationship between the age of sexual maturation and the age at death in Novaya Zemlya reindeer. Abscissa: number of survived winters; ordinate: the winter by which sexual maturity was attained (after Klevezal and Sokolov, 1999).

*Age of sexual maturation.* Determination of the age of sexual maturation in animals that died from natural causes makes it possible to study the relationship between the rate of sexual maturation and the life span. It turns out that among the Novaya Zemlya reindeer, the late maturing animals lived for a long time, unlike the early maturing ones (Fig. 2) (Klevezal and Sokolov, 1999). This confirms the suggestion that delayed sexual maturation decreases the cost of reproduction and increases the life span (Stearns and Grandall, 1981).

*Time of birth.* Estimation of the proportion of individuals born at the end of the breeding season to reindeer of different ages from the Novaya Zemlya population and Taimyr population (taken for comparison—Klevezal and Sokolov, 1997) has provided us with the following results (Fig. 3). Among young Novaya Zemlya reindeer that died from natural causes, most were born late, while among shot Taimyr reindeer, there were fewer animals that were born late. This confirmed the conclusion from direct observations on calves: the survival rate of late-born individuals was lower during



**Fig. 3.** Proportion of late-born animals in reindeer of two populations at the age of one to two years (□) and five to eight years (▨), %.



**Fig. 4.** The length of the mandible diastema in Novaya Zemlya reindeer (ordinate, mm) that died at different ages (abscissa, years): (●) males, (○) females (after Krushinskaya *et al.*, 1997).

the first summer of life (Adams *et al.*, 1995). However, there were late-born animals among the deer ranging in age from five to eight years. Both died from natural causes or were shot but their proportion was much less. The presence of late-born animals among the young Taimyr reindeer that were shot, and a decreased proportion of such animals in the older age group suggest that these reindeer may survive not only the first but also subsequent winters, but they have less chance of surviving to the age of five to eight years than deer born during the period of mass reproduction. Their survival rates are different in different populations and in the same population in different years and appears to depend on environmental conditions (Klevezal and Sokolov, 1997).

**Body size.** Finally, data on the age of animals make it possible to use the mandible size not only for sex

determination (if there are sex-specific differences in body size), but also for characterization of the total size of an animal at the moment of its death. The mandible length is a good indication of the skeletal size of the deer (Suttie and Mitchell, 1983) and is highly correlated to the body mass (without fat and viscera) (Lowe, 1972).

When analyzing bone residues from animals that died long ago, it often turns out that the mandible ramus is damaged and precise measurement of the mandible is impossible. In this case, the mandible diastema length may serve as an indication of the reindeer's body size (Bertoille and Decrombrugge, 1995). This index is highly correlated with the condylobasal skull length (according to Empel (1962), in European bisons aged seven years or higher,  $r = 0.93$ ,  $n = 25$ ) and to the mandible length (in adult reindeer of different populations,  $r = 0.94$ ,  $n = 50$ ; calculated from the measurements of N.L. Krushinskaya).

In reindeer older than three years, gender can be identified according to the diastema length (Krushinskaya *et al.*, 1997), and the growth of males and females of a given population can be characterized when compared with the age. For example, it can be seen in Fig. 4 that: (1) the growth of males actively continued after four years, while that of females was delayed, and (2) the oldest individuals in the sample were far from being the largest, which is especially distinct for males. The first conclusion confirms that the diastema length can be used for the growth characterization, since similar differences in the growth rate of males and females were found when analyzing the age related changes in the body mass (Nieminen and Petersson, 1990). The second conclusion is more interesting. It provides materials for analysis of the ratio of mortality rate to total body size in conspecific individuals. This ratio has so far been discussed on the basis of interspecific comparisons (Promislow and Harvey, 1990).

## CONCLUSION

Thus, detailed analysis of the mandibles and teeth collected on Novaya Zemlya from reindeer that died as a result of natural causes made it possible to examine the relationship between the life span and total body size, age of sexual maturation, and time of birth and also caused us to search for causes of increased mortality at the young age.

In addition, teeth and bone tissue accumulate different chemical substances (Sharon, 1988), while the tooth enamel records the external irradiation (Ikeya *et al.*, 1984; Pass and Aldrich, 1985). Chemical and physical studies of teeth and bone tissue allow the estimation of the level of individual contamination and, together with investigation of the recording structure, elucidation of the dynamics of contamination and its effect on the state of individuals in a population (Klevezal *et al.*, 1997; Klevezal and Sokolov, 1999).

Thus, the effect of environmental contamination on the mortality rate in populations can be analyzed provided there are well equipped chemical and physical laboratories.

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