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AN EXPERIMENTAL APPROACH TO THE SPECIES PROBLEM

ЕКСПЕРИМЕНТАЛЬНЫЙ ПОДХОД К ПРОБЛЕМЕ ВИДА

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During 20 years our laboratory carried out investigations on the pathways of adaptations of animals to different life conditions. More than 100 species of mammals, birds, reptiles and amphibians were examined. Our investigations involved morpho-physiological indices, metabolic rate, vitamin contents of tissues, endocrinological differences, activity of some digestive ferments, tissue respiration, protein thermostability, haematological indices, immunological reactions, etc.

It has been established that every adaptation to life conditions which requires intensification of metabolism calls for expressed morpho-physiological alterations. But the higher the degree of adaptability to that complex of conditions which requires increased energy expenditure, the less expressed are the morpho-physiological adaptations. This statement could be now confirmed by a large number of observations. Mountain populations of the widespread species *(Clethrionomys rutilus, C. glareolus)* are characterized by relatively increased heart weight. The specialized mountain species *(C. frater, Ochotona alpina)* do not differ essentially from the plain species. Quite analogous results have been obtained by the comparison of subarctic populations of *Microtus* ssp. with the lemmings; subarctic populations of widespread species of birds with specialized arctic species. These observations are summarized in two monographs (S c h w a r z 1959, 1969) and a large number of articles. Therefore I can confine myself only to general deductions.

Each innate feature has an energetic cost. We define this notion as the amount of energy that is necessary to maintain the existence of animals under concrete life conditions. Morpho-physiological adaptations are perfect from the functional viewpoint but their energetic cost is high. Therefore natural selection favours the development of adaptations which make it possible to keep up the energetic balance by means of biochemical adaptations, adaptations on the tissue level. The biochemical tissue adaptations can be of very general importance (the degree of protein lability, alterations in interrelationships between electrogenesis and mechanogenesis in the heart muscle, alteration in tissue respiration or protein thermostability) or of very specialized character (increased resistance of tissues to dehydratation in desert forms, decreased O_2 requirement of cells in mountain animals, *etc.*).

Accordingly, the highest degree of adaptation is not accompanied by pronounced morphophysiological alterations. As an example, let us cite one of the recent works. M a n n (1968) writes: "Der höhenbedingte, stark verminderte O_2-Gehalt der Atmosphäre erfördert wirksame Anpassungen ... Seltsamerweise konnten diese Anpassungen aber keineswegs bei den eigentlichen Höhenformen festgestellt werden" (Biogeography and Ecology in South America, The Hague, 1968). The author is astonished that specialized species do not possess any morpho-physiological adaptations to the mountain life. Our hypothesis makes it clear that there is no reason for astonishment. The observations of M a n n demonstrate one of the more general laws of animal evolution.

All animal species without exceptions are characterized by a higher degree of adaptability to that complex of conditions which led to their origin than is any specialized intraspecific form. To this law there is no exception. This means that any animal species, during its evolution, passed a stage of subspecific specialization, featured by rounding out morpho-physiological adaptations. Later stages of microevolution culminated in the reduction of energy economy by those adaptations which finally elicited differences at the tissue level, *i. e.*, leading to tissue incompatibility, and to reproductive isolation. It was very interesting to prove these conclusions by experimental investigations. A fairly large number of related forms were studied under natural and experimental conditions: *Microtus oeconomus oeconomus* Pall., *M. oe. chahlovi* Scalon, *M. gregalis gregalis* Pall., *M. g. major* Ogn., *M. juldaschi juldaschi* Sev., *M. j. carruthersi* Thomas, *M. middendorffi middendorffi* Poljakov, *M. m. hyperboreus* Vinogr., *M. arvalis transuralensis* Serebr., *M. transcaspicus* Satunin, *Lagurus lagurus* Pall., *Lemmus obensis* Brants, *Clethrionomys frater* Thom., *C. rufocanus* Sundev., *Alticola roylei* Thom., *A. strelzovi* Kastsch.

The following are the most important indices used in our investigations: morphological features commonly used in taxonomic procedure, ecological patterns, morpho-physiological indices, electrophoresis of blood serum, immunological reactions, karyology, experimental hybridization. Thus we create the necessary preconditions for investigating the species problem from different viewpoints. I have no possibility to analyze the results of our work in detail and shall dwell only on some most important questions.

Between neighbouring populations of *M. arvalis, M. oeconomus* and other species no morpho-physiological differences could be observed. The differences in electrophoregrams were insignificant, but between all the populations highly significant immunological differences were found *M. oe. oeconomus* differs from the tundra dwelling form of the same species *(M. oe. chahlovi)* in the tail length and some important skull dimensions. The major physiological difference consists in increased fecundity of the northern subspecies. Some morpho-physiological peculiarities indicate a decreased metabolic rate of *M. oe. chahlovi.* The hybrids of the forms are fertile and the electrophoretic and immunological differences do not

surpass the corresponding differences between neighbouring populations.

The morphological and ecological differences between steppe and tundra subspecies of *M. gregalis* are by far better pronounced. Suffice it to say that competent zoologists consider *gregalis* and *major* as good species. The hybrids are fertile, the electrophoretic distance is greater than that between other intraspecific forms but incomparably less than between species.

M. middendorffi and *M. hyperboreus* are commonly considered as separate species. Experiments have shown clearly that this point of view is a mistake: *hyperboreus* is a mountain subspecies of *middendorffi.* No important morphological differences between these forms could be observed. The only one is the colour polymorphism of *hyperboreus.* The hybrids are absolutely fertile. Karyograms are identical (NF for males = = 57–59, for females = 58–60; a polymorphism of an autosome is expressed).

Quite another situation can be illustrated by a comparison of *M. j. juldaschi* with *M. j. carruthersi.* These forms are also considered as separate species. This point of view could be encouraged by karyological investigations. Karyological differences between *juldaschi* and *carruthersi* correspond to the differences between species (morphology of the X-chromosome and 3 pairs of autosomes). In spite of this, *juldaschi* and *carruthersi* give fully fertile hybrids. Morpho-physiological and ecological differences between these forms are unimportant and the differences in electrophoretic patterns are of a subspecific rank.

The taxonomic status of *M. transcaspicus* and *M. arvalis* is doubtful. Some zoologists regard them as species, others as subspecies. Important morphological and physiological differences were found to exist between these forms. The electrophoretic distance is of the rank of good species. Breeding experiments demonstrate that the fertility of pairs "*transcaspicus* × *arvalis*" is reduced, the hybrid animals are sterile.

All in all, we come to the conclusion that the determination of the taxonomic status of related forms must be based on a background of complex investigations. In contrast to any intraspecific forms, a species is biologically independent. From the genetic viewpoint it is that system of integrated genotypes whose enrichment by genetic material from other analogous systems would reduce its vitality. This defines the evolutionary independence of the species. At the genetic and morpho-physiological levels various forms of reproductive isolation occasionally appear as phenomena of another order. Therefore "species specifics" are manifested only at evolutionary and ecological levels. Artificial specific criteria, though based on newest research methods, must be only regarded as methods in the true sense of the word.

But new methods can be very useful in investigating some particular questions of the species problem. Of particular interest are the methods

of protein taxonomy, especially the immunogenetic techniques. It is generally known that the success or failure of a tissue graft to grow in a recipient depends upon the genetic similarity between the donor and the recipient. Therefore in our laboratory we carried out special transplantation experiments, combined with the investigation of the antigenes of the erythrocytes. Several intraspecific forms of *M. arvalis, M. oeconomus* and *M. gregalis* were studied. It was found that on the background of distinct interdeme differences, species = specific reactions on a heterotransplantate were observed in every experiment without exception. Every subspecies, every form, every population distinguishes its own species from an alien one. Unity of a species on tissue level combine all the ranks of intraspecies differentiation. This was confirmed both by the common degree of tissue incompatibility on heterotransplantate (independently of the intraspecific differences of the donors), and by the species specific of the humoral reactions. Immunological investigations can help us to understand some phenomena concerning the species problem, namely the origin of sibling species, and the origin of a hiatus between related species.

Neighbouring populations under similar life conditions acquire, under the influence of stabilizing selection, common phenotypes. But the genetic structure of independent populations cannot coincide in details. Under these conditions a stabilizing selection on an optimal phenotype inevitably leads to gene rearrangements, necessary for the neutralization of new mutations. This process results in the origin of genetic differences between populations of the greatest degree of morpho-physiological similarity. As was mentioned above, this was proved experimentally: the degree of genetic differences does not coincide with the degree of morpho-physiological differences. In extreme cases the genetic differences between indistinguishable forms may result in genetic incompatibility, and genetic isolation. An establishement of sibling species is completed. The origin of "normal" species is governed mainly by directional selection, the origin of sibling species, by stabilizing selection. On the other hand, methods of taxonomy can be of great importance in the analysis of the hiatus problem. It is generally known that in natural populations great genetic variability is masked by well-known phenotypic mechanisms (cryptic variability). But investigations carried out with the aid of modern methods demonstrate the true range of variability in natural populations. It was established that the mean immunological differences between different populations usually do not surpass the corresponding differences between extreme variants in one population. Having in mind the interrelationships between biochemical and morphological characters of animals, we can state that in this situation a creation of a hiatus within a species is impossible. A formal criterion of a species (hiatus) which was established long time ago by practical taxonomists reflects its biological essence, the genetic unity manifested in the nature of intrapopulation variability on biochemical level.