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ANOMALIES IN NATURAL POPULATIONS OF AMPHIBIANS: A GENERAL SURVEY AND METHODOLOGICAL RECOMMENDATIONS FOR STUDY

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The article presents a methodological review and analysis of the occurrence and nature of the anomalies in populations of amphibians. possible methodical and methodological approaches of researches in this direction were discusses.

Статья представляет собой методологический обзор и анализ встречаемости в природе аномалий в популяциях амфибий. Обсуждаются возможные методические и методо-

логические подходы при проведении исследований в данном направлении.

Monsters and anomalies

The term «monster» comes from the Latin name *monstrum*, from the Latin verb *monstro*, *monstrare*, «to show». A monster is something that one shows, because it is unusual, strange or frightening. The term conveys a wide variety of meanings.

Anomalies and monstrosities have long interested humans, since the antiquity. Of course, the interest was greatest for human «monsters»: albinos, twin monsters, synophthalms, etc. They were often interpreted as punitions or messages from the gods, maledictions, consequences of transgression of human laws (e. g., incest), of traumatic events in the life of the mother, of developmental troubles or so-called «atavism».

Who says «monster» also says «non-monster» or «normal». But what is «normal» in biology? Biology has now left its «essentialistic» stage behind. Organisms and species are no more viewed has expressing an «essence», but as resulting from a permanent process of evolution. And evolution is possible only because there exists within each species and population a variability, a polymorphism. According to this point of view, «deviants» from the norm should not be considered as «monsters», but as an expression of the natural variability of organisms.

Variation in natural populations

The progress in studies of anomalies followed several steps: (1) description of isolated cases (considered anecdotal), inventory and classification of anomalies; (2) search for anomalies in natural populations; (3) search for the causes of anomalies, in particular through the production of abnormal individuals by biologists.

The work of inventorying and describing monsters was the purpose of descriptive teratology, a discipline which developed in the 19^{th} and 20^{th} centuries.

The search for the causes of anomalies resulted in the distinction between two main categories of causes of anomalies: (1) genetic causes (genotypic anomalies), such as exceptional genetic mutations or composite organisms (hybrids); (2) non-genetic causes (phenotypic or epigenetic anomalies), resulting from traumatisms during development, physico-chemical aggressions (e. g., by pesticides or radiations), biological aggressions (e. g., by hormones or viruses), parasitism, or composite organisms (chimeras). But the distinction between the two categories is sometimes not straightforward. For example, some violent aggressions (mostly by radiations or by some chemicals) may entail mutations in the DNA of organisms submitted to them, but the result will be different according to the cells concerned: mutations in somatic cells may induce anomalies, but these are not transmissible to the offspring, whereas mutations in gametes or their primordial cells will be transmitted to the offspring, which will have very different consequences on the populations concerned. In the first case we will speak of «teratogenic factors», and in the second one of «mutagenic factors», although in both cases the distal cause was the same, a violent aggression on the DNA of the cells, and both often occur together.

Qualitative variation results in the regular appearance within populations or species of rare phenotypes (albinos, polydactyls, cyclops, etc.), whereas quantitative variation in measurable or countable characters results from the existence in each population and for each character of a curve of variation which often follows a «normal» law (the Gauss bell curve), with some «extreme» individuals appearing very deviant.

Terms are important in science and a few words about terminology are in order here. Among the various terms that have been used to describe «monsters», some are preferable. The terms *monstrosities* and *monsters* should be avoided as they carry the teleological notion of «mistake of nature». The terms *deformities* and *malformations* carry the notion of «form» and are too restrictive, because they exclude variants in coloration, developmental chronology or sex characters. The terms *mutations* and *mutants* carry the notion of genetic transmission and are therefore not appropriate for phenotypic anomalies. The terms *anomalies*, *abnormals* or *deviants* are to be preferred because they are the most neuter and general, being just descriptive and carrying the notion of «normality» (which also implies the recognition of some individuals as «normal»).

Intraspecific variation within populations or species, mostly due to genetic causes (although these are usually also moderated by epigenetic factors), is called *polymorphism* (a misleading term also, as it seems to refer only to form, although it can also concern other characters such as coloration). Each population displays an internal variability, so where should be placed the barrier between polymorphism and rare mutation? The reply is simple: there is no such barrier; this is only a matter of convention. Population biologists usually admit that an allele present at a frequency below 1 % in a population is not considered as polymorphism but as an exceptional anomaly or mutation, which is not maintained over generations in the population but can occur again at each generation. But in fact, in most studies of natural populations, the samples studied are usually too low (below 100 specimens) for allowing to ascertain such a rate, so the convention has moved to 5 % for purely practical, not theoretical, reasons. However, some natural anomalies (such as the anomaly P in European green frogs), due to aggressions to which the animals were submitted, can be present, in some populations and some years, in high proportion (up to 70 %). The same «anomaly» that may be present in less than 1 % of the individuals in one population may be present in 20 % of the specimens in another population. Therefore the separation between «*anomaly*» and «*polymorphism*» results from an arbitrary convention.

Studies of anomalies in amphibians

Amphibians are a privileged material for the study of anomalies. They are *v*ertebrates (like mammals and man), of a good size (neither too small not too large), which usually occur in large populations, usually have a high fertility, and lay large and easily accessible eggs and embryos. They have therefore been used, since the end of the 19^{th} century, in innumerable studies which have followed all the way from descriptive embryology to evo-devo, through experimental embryology and developmental genetics.

The descriptive embryology of amphibians has allowed understanding in all their details the processes of gametogenesis, fertilisation, and the main stages of embryological development.

The experimental embryology and teratology of amphibians developed with the idea that producing monsters and studying them would allow to understand the processes of ontogeny. An anomaly derives often from a «mistake» in development, so that understanding this mistake may help us to analyse the «normal» development. Experimental teratology therefore developed as the science of «producing and understanding monsters». For this purpose it made use of a wide variety of techniques, including grafts, chimeras, hybridization, transfer of a nucleus from a cell to an ovum, injections, etc.

The relationships between anomalies and evolution, although very interesting, have been less explored, probably because for a long time the specialists of the two questions had different cultures and little contacts, but this is now changing. It is striking to note that, what is an anomaly in one species, genus or family, will be the norm in another group. For example, the blue coloration of body, which occurs as exceptional phenotypes in Eurasian ranids and hylids, is the norm in some tropical species, e. g. of dendrobatids. Translucent belly, which results from rare pigmentary mutations in all groups of amphibians, has been «fixed» as the norm in some groups like the centrolenids. Ectrodactyly (absence of digits), which is a rare mutation in many amphibian species, has been «fixed» as the norm in some species, e. g. *Brachycephalus tridactylus*.

Of particular interest are the developmental anomalies usually called heterochrony but that should rather be called aneuchrony (abnormal chronology of development) because aneuchrony can be either heterochronic (with dissociation between traits, like in neoteny or paedogenesis) or homochronic (with a simple synchronic acceleration or slowdown of the tempo of development of all characters). Rare anomalies involving aneuchrony include adult anurans having kept their larval tail or coiled intestine, or one arm still below the skin. More common are the cases of reproduction of specimens having kept their branchiae and many other larval characters, but the gonads of which have become functional, a phenomenon which has become «fixed» in some populations, species or groups of Urodela but which does not exist in Anura.

Pigmentary anomalies (albinism, white frogs; melanism, black frogs; flavism, yellow frogs; «caeruleism», blue frogs; translucent skin; etc.), which on the whole are rather frequent in amphibian populations, are due to the absence of some pigmentary cells or of some pigments in the skin or iris of amphibians. They are usually caused by simple (monogenic) genetic determinisms, but external (virus) or internal (hormonal) factors can also interfere with pigmentation. A phenotype which is quite common in many populations of amphibians is that of the «black eyes», due to the absence of iridiophores in the iris, and usually caused by a single recessive mutation.

Another large and diversified category of anomalies touches the structure and characteristics of limbs and digits. Rather common in natural populations are the polydactyly (supernumerary digits), ectrodactyly (missing digits), syndactyly (fused digits) and clinodactyly (bent digits), but others do exist. All these anomalies can have both genetic (mutations) and various non-genetic proximal causes. In contrast, the anomalies concerning the whole limbs, such as polymely (supernumerary limbs) or ectromely (missing limbs), rarely have genetic causes, being usually due to various kinds of aggression on the organisms.

Anomalies as warning signals

For a long time, the study of amphibian anomalies has been the matter of rather few studies, because it appeared «anecdotal» and of little interest to many biologists. In the recent decades however, the attention has been called on several cases of mass anomalies in natural populations of amphibians, and anomalies have become to be seen as warning signals regarding the «health» of amphibian populations, and by way of consequence, as indicators of environmental health and potential human hazards. This is justified, in view of the fact that, during the successive phases of their life cycle, amphibians, whose naked skin makes them particularly sensitive to some aggressions, occupy several habitats where they can be in contact with various pollutants and other perturbating factors. For this purpose, it is of prime importance to distinguish between the various causes of anomalies, and above all between genetic and epigenetic anomalies. Such distinctions are important for prospective evaluation of the fate of the populations concerned: somatic mutations caused by teratogenetic factors will not be transmitted to the offspring, whereas gametic mutations caused by mutagenic factors will or may be so, sometimes through complex mechanisms. After a long period of lack of interest, amphibian anomalies have suddenly arisen high level of media attention, perhaps too much, because some of the researchers interested in these mass anomalies lacked the necessary background to interpret correctly the facts. A number of studies published in the recent years on these questions contain some methodological flaws, such as absence of distinction between a correlation and a cause, between genetic and non-genetic anomalies, between teratogenic and mutagenic factors, etc. It is therefore appropriate to provide a few warning words in this respect.

Methodological recommendations and warnings for the study of anomalies in natural populations of amphibians

Field survey

(1) Study as many localities as possible in an area (not only those where abnormals were found).

(2) Study all amphibian species in each locality (not only the «target» species).

(3) Always practice random sampling (do not look specially for abnormals).

(4) Study numbers of specimens as large as possible (at least 100 if possible, preferably above 1000). Night survey by teams of researchers are more efficient in this respect.

(5) Note everything: the number of individuals of each stage and sex examined; all phenotypic anomalies, including «tiny» anomalies and apparently «accidental» ones (wounds); make detailed descriptions, photographs, sketches, etc.

(6) Keep alive and bring to the laboratory all «interesting» specimens.

(7) Release all others on spot of capture only after having examined them all (which requires equipment for stocking specimens alive in good conditions for a while in the field).

(8) Survey the same locality regularly, over years or decades if possible, to obtain data on the trends of prevalence and nature of anomalies over long periods.

Search for the causes of the anomalies

Detailed phenotypic study of all abnormals

(1) Harmony (result of spontaneous development, not of wounds).

(2) Particularities of the phenotype (which may be specific of some syndromes).

(3) Bilaterality and symmetry (which usually eliminates the possibility of a random cause such as wounding by predator).

(4) Gradients of severity: postero-anterior, axial-postaxial, proximo-distal (specific of some syndromes).

(5) Anomalies associated in syndromes (variability within syndrome).

(6) Detailed phenotypic study of all abnormals.

(7) Association of anomalies that do not constitute a syndrome, in individuals and in populations.

(8) Similar anomalies in sympatric species.

Cautions in phenotypic study: (a) beware of phenocopies; (b) a correlation is not a cause; (c) beware of statistics: probabilities may be misleading; (d) a cause must be demonstrated (e. g., by reproducing an anomaly in controlled laboratory conditions) before being accepted as responsible for the phenotypes and phenomena observed.

Breeding and crossings

Breeding of abnormal individuals in captivity can be very informative. For example, some abnormal colorations tend to change with time, sometimes over years.

Crossings should use artificial fertilisation. They should be done between abnormals, between abnormals and normals, and between normals. Following standard methodological procedures in crossing studies, the gametes of each individual should be divided into several samples and these samples used in several crosses, including controls (involving only normal individuals). As many mutations are recessive, in many cases clarification of the genetic cause of an anomaly will require back-cross of the offspring among themselves or with the parents, therefore facilities for long-term breeding of amphibians.

Cautions in crossings: (a) beware of phenocopies: both in pigmentary anomalies (albinism, melanism, etc.) and in digital anomalies (polydactyly, ectrodactyly, etc.), similar anomalies may result from different mutations or from other causes; (b) beware of variability and incomplete expression (variation in penetrance).

Experimental parthenogenesis and gynogenesis

Experimental parthenogenesis and gynogenesis, associated with techniques duplicating the number of chromosomes before the start of development, allow to obtain diploid offspring homozygous for all alleles, and therefore to discover, in one generation only, recessive alleles that may be responsible for some anomalies.

Cautions in parthenogenesis and gynogenesis: the ploidy of the offspring should be checked (by cytogenetic or other methods) in order to ascertain that these specimens are indeed diploid.

Regeneration experiments

Carried out in tadpoles of Anura, or in Urodela at all stages of life, they may allow to show that the factor responsible for a digit or limb anomaly is no more active in an individual, and therefore was not a genetic factor: for example, a polydactylous limb may regenerate normal. Such experiments should be carried out on a single side of body in specimens showing a bilateral limb anomaly.

Caution in regeneration experiments: beware of hyperregeneration, a common phenomenon in amphibians after amputation.

Survey of potential external factors in the habitat

The following list of factors potentially involved in anomalies in amphibian populations is in no way restrictive: sunlight; temperature; oxygen; pH; radioactivity; chemicals; fertilizers; hormones; viruses; bacteria; parasites; predators… Of course, they cannot (and should not) be studied in all localities, but if there are clues for possible unusual particularities in the water or aspect of the habitat they should be explored in detail.

Caution in survey of external factors: (a) a correlation is not a cause; (b) beware of statistics: probabilities may be misleading; (c) a cause must be demonstrated (e. g., by reproducing an anomaly in controlled laboratory conditions) before being accepted as responsible for the phenotypes and phenomena observed.

Conclusion

In amphibians, anomalies observable by external survey are very diverse and may touch many parts of the organism (limbs, head, body). But, like in all animals, the development of amphibians is very constrained at each stage by the ontogenetic stages already covered. After a given stage in development, the number of possibilities offered to an embryo is limited. After the stage blastula, an embryo roughly has the «choice» only between gastrulation, exogastrulation, or degenerescence and death. The same applies, with varying degrees of freedom, at all stages of development and for all organs: a hand can have four fingers (normal number in most amphibian species), three fingers (ectrodactyly), five fingers (polydactyly), but certainly not 30 fingers. The ontogenetic possibilities being limited, similar phenotypes can result from different causes (phenocopies). Therefore, except in rare cases, the mere examination of the phenotype is not sufficient to establish the cause of an anomaly. This word of caution (beware of phenocopies), as well as a few others (a correlation is not a cause; probabilities may be misleading; a cause must be demonstrated; etc.) must be kept in mind for studies aiming at ascertaining the causes of anomalies in natural populations of amphibians, with potential consequences the fate of these populations as well as on the environment as a whole.

THE ANOMALY P IN PALAEARCTIC GREEN FROGS OF THE GENUS *PELOPHYLAX* (RANIDAE)

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An aritcle describing history and specific of anomaly P syndrome. So called «anomaly P» first discovered by Jean Rostand. He found that polydactyly was just a mild form of a complex syndrome which included much more severe anomalies, such as very high numbers of toes and fingers, brachymely or oedemas in the inguinal region. He gave the name of «anomaly P» to this syndrome and