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

started to study its particularities and to try to understand its determinism. The anomaly P was found or suspected to occur in many other populations of green frogs (Pelophylax) of the Palearctic region. Rostand supposed that the anomaly P related with an infectious agent, which could be a teratogenic virus. The hypothesis agrees with the data published so far, but this factor has not yet been isolated. The syndrome involves facts of abnormal cellular multiplication and tissue differentiation and growth.

Статья описывает историю и специфику аномалии Р. Так называемую аномалию Р впервые обнаружил Жан Ростан. Он установил, что полидактилия – это легкая форма сложного синдрома, который включает более сложные отклонения: очень высокое число пальцев конечностей, брахимелию и отеки паховой области. Он дал название этому синдрому: «аномалия Р». Аномалия Р, как предполагается, встречается в популяциях ряда видов палеарктических зеленых лягушек (*рода Pelophylax*). *Ростан полагал, что аномалия Р связана с инфекционным агентом, который может быть тератогенным вирусом. Синдром включает в себя феномен аномальной клеточной пролиферации, дифференцировки и тканевого роста.*

Discovery of the anomaly P

The discovery of the anomaly P is an interesting and informative chapter of the history of science, which shows that research, if left to the freedom of researchers and not prisoner of «programs» imposed from above or outside, can lead to unexpected discoveries. The French biologist Jean Rostand (1894–1977) was interested in the phenomena of «reproduction by females alone», i. e., parthenogenesis. He used frogs to try to obtain embryos from unfertilized eggs. He first repeated Eugène Bataillon's (1864–1953) experiences on traumatic parthenogenesis, in which the egg is triggered to start its development by a small glass needle, but this technique is very heavy to use, because only a small proportion of eggs develop successfully. Rostand then used a much more efficient technique, that of gynogenesis, in which the egg's development is induced by an inactivated (irradiated) sperm, whose genetic material does not enter the egg and does not participate in the development. Such unfertilized eggs develop harmoniously only if the second polar body resulting from

the meiosis is reincorporated into the egg to make it diploid before the start of the divisions. The embryo then turns out to be homozygous for all its alleles (except for *de novo* mutations). Rostand then observed that a high proportion of the imagos (just metamorphosed froglets) that resulted from gynogenesis were showing pigmentary (such as albinism) or morphological (such as polydactyly) anomalies. This was the result of having rendered homozygous some rare recessive genes which seldom express themselves in natural populations, because they rarely happen to be homozygous. Rostand then was then the first to realise that gynogenesis was a technique allowing to explore the genetic heritage of individuals in frogs, and he started building a discipline which was then new, the «genetics of batrachians». This prompted him to go out of his laboratory and to start exploring natural populations of amphibians, with the help of many correspondents from all over France. He then discovered many natural pigmentary and morphological anomalies in several common species. He made crossings involving these abnormals and was then able to show that some of these anomalies, such as «black eyes» in green frogs or polydactyly in the common toad, were determined by recessive or dominant mutations.

In this process, he discovered a new kind of polydactyly in green frogs (now the genus *Pelophylax*), touching sometimes only the hind limbs but sometimes also the fore limbs, which was quite common in some populations. When he studied the tadpoles in these populations, he realised 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 started to study its particularities and to try to understand its determinism. This work occupied much of his time in the last years of his life, but he was not able to bring it to its conclusion, and today the mystery of the anomaly P is still unsolved, because no laboratory yet decided to devote the appropriate means to trying to solve it.

The anomaly P syndrome

The mildest form of the anomaly is polydactyly (presence of supernumerary digits). This polydactyly shows a postero-anterior gradient:

in all abnormal specimens, the hind limbs are touched by polydactyly (from 6 to 9 toes), whereas the fore limbs are touched only when polydactyly on hind limbs is strong (more than 6 toes). Other morphological peculiarities of this syndrome include a good, although not always perfect, bilateral symmetry: i. e., the number of additional digits is always roughly the same on both sides, with sometimes one more toe or finger on one side, but never an excess of several digits on one side (e. g., 6–7, 7–7 or 7–8 toes, but never 6–8). The first additional toe is usually longer than the «first» normal toe, which gives the feet of these frogs a very special aspect, quite different from that of usual polydactyly in other amphibian species. But this polydactyly is only the weakest form of a very polymorphic syndrome, which can take much more severe forms in tadpoles, including stronger polydactyly (up to 15 toes), polymely (presence of small supernumerary hind limbs), brachymely (shortened limbs), and various types of bony excrescences and tumors, especially in the inguinal region. These severe manifestations of the syndrome have an impact on the survival of the individuals: most affected tadpoles die during or just after metamorphosis, mostly from internal haemorrhages due to mechanical problems caused by their supernumerary or malformed bones, so that only the mildest forms of the syndrome (simple polydactyly) are present in the adults.

Frequency in populations and ecological impact

The anomaly P may touch a very high proportion of the larvae in a population, but most of them do not survive metamorphosis, which explains why the rate of anomalies is much higher in tadpoles than in adults.

At the scale of a whole population, the anomaly shows a strong variability over time, being very frequent some years and very rare or absent in other years, with a possible return after several years of absence. Observed variations in frequency of anomaly P range from 0 to 80 % in tadpoles and from 2,6 to 14,5 % in adults, from 1949 to 1967 in the Trévignon population from Brittany, and from 14 to 70 % in tadpoles, from 1961 to 1970 in the Lingé population from Indre. The frequency of the most severe forms, lethal at metamorphosis, is also variable from year to year (e. g., from 26,9 to 47,1 % in Trévignon from 1952 to 1958),

but is not higher when the frequency of abnormal specimens is higher. The frequency of the anomaly is also different, within the same year, at different dates, and seems to grow when the temperature in the ponds gets higher.

First discovered by Rostand in a Brittany pond, the anomaly P was found or suspected to occur in many other populations of green frogs of the Palearctic region. The complete syndrome in adults and larvae was observed in several regions of France, in the Netherlands and Morocco. Mass polydactyly, or polydactyly which on morphological grounds closely resembles that of anomaly P, was reported from various other regions of France and from Austria, Belarus, Germany, Switzerland and European Turkey. Finally, isolated cases of polydactyly, possibly also belonging in anomaly P, were reported from France, Germany, Greece, the Netherlands, Poland and Russia.

The search for the cause

All crossings between specimens affected by anomaly P produced only normal offspring, even if both parents were polydactylous, some of them severely.

In a crucial series of experiments, Rostand showed that amputation, in young tadpoles, of the distal part of a hind limb touched by anomaly P, was followed by regeneration of a normal limb, which demonstrated that the teratogenic factor, whatever it may have been, had stopped being active at the time of regeneration. Such a result, associated with those of the crossings, strongly suggested that the anomaly was caused by an exogenous teratogenic factor having a precocious and temporary action on the limbs of tadpoles. As many such factors could be suspected, Rostand carried out varied experiments in the hope of reproducing artificially, in the laboratory, polydactyly and the other anomalies observed in the anomaly P syndrome. However, he did not succeed in reproducing such anomalies by the action of various chemicals, by raising eggs and larvae in brackish water or in water taken in the ponds where the anomaly was present, by the action of various physical shocks including thermal shocks, long exposure to high or low temperature or to sun, dehydration, etc., or in provoking overmaturity of egg before fertilization.

Artificial radioactivity linked either to civil or military human activities can be dismissed as the possible cause of anomaly P, as isolated cases of the latter have been reported since the beginning of the 19th century, and mass occurrence of polydactyly clearly now referable to anomaly P has been known since before the Second World War (1937).

All these observations led Rostand to think that the anomaly P was not due to a chemical or physical agent, and to favour the hypothesis of an infectious agent, which could be a teratogenic virus, and which would be active between hatching and the appearance of the hind limb buds.

At the end of his research activity, Rostand reported a series of experiments realised first in the field and then in the laboratory. Tadpoles raised in large cages directly submerged in a pond where the anomaly P was present, and with fishes (tenches and eels) collected in this pond, showed severe anomalies belonging to the anomaly P syndrome, whereas tadpoles in cages without fishes did not show any anomaly. The sensitive period for the induction of anomalies in such conditions turned out to be limited to the first days of free larval life. Finally, Rostand reported having obtained highly abnormal specimens in the laboratory, by feeding just hatched tadpoles with intestinal contents of fishes from the pond, which suggested that the factor responsible for the anomaly P seems to be present in the digestive tract of such fishes. The hypothesis that it could be a teratogenic virus agrees with the data published so far, but this factor has not yet been isolated.

On several occasions, rather heavy experimental protocols, involving the comparison of control tadpoles with tadpoles fed with fish intestinal contents, either intact or submitted to ebullition, freezing, filtration or chromatographic fragmentation, did not give any results, so prudence is in order, in particular since the last works of Rostand were made in collaboration with an unreliable «amateur» lacking scientific training and now known to have made methodological mistakes, if not deliberate frauds, in the protocol of some works.

As of today, the teratological virus appears the best hypothesis to account for the various observations summarized above, but it is still not demonstrated. If it proved true, and if this virus could be isolated,

it would provide research with a very powerful teratogenic factor, which could throw some lights on problems of cell proliferation and differentiation, with possible impacts on research on cancers and other pathologies that imply uncontrolled cell multiplication.

Ecological significance

Whatever its causes, anomaly P is an important phenomenon for the populations of green frogs where it occurs, as it reduces the survival of frogs. Before metamorphosis, the survival of strongly abnormal larvae does not seem to be affected, and artificial or spontaneous prolongation of the larval condition allows to keep these specimens alive very long, up to one year and a half. However, even in protected conditions in captivity, specimens exhibiting the most severe forms of the anomaly (with brachymely and various supernumerary bony formations) all die within the first weeks after metamorphosis, merely because of the internal injuries caused by the bony excrescences in the hind limbs. Thus, for purely mechanical reasons, the anomaly in its severe forms is totally lethal at metamorphosis or just after, and only the frogs showing the benign form of the anomaly (simple polydactyly) do survive. Since the frequency of the severe forms often reaches 40 or 50 % of the abnormal specimens, which in turn may represent up to 80 % of the tadpoles of a population, in some cases about 40 % of the tadpoles of a given pond may die at metamorphosis. Such a mortality rate, especially if repeated for several years, might have a significant impact on the dynamics of these natural populations. However, until now no study has been devoted to this question.

Species affected

Most of the observations on the anomaly P summarised above were done before the discovery that Palearctic green frogs consist of several «normal species» and of several *kleptons*, i. e., particular species derived from hybridization between two «normal species» but with a particular meiosis that allows the maintenance of «first generation hybrids» over many generations. Therefore it is currently unknown which of these species and kleptons are touched by the anomaly, but the distribution

of the latter is large enough to be sure that it concerns at least several taxa, if not all European green frogs, currently placed in the genus (or subgenus of *Rana*) *Pelophylax*. Currently, 20 species and 3 kleptons are recognized by taxonomists in this genus, among which 9 species and 3 kleptons occur in Europe and northern Africa. From the distributional data on anomaly P, at least the species *Pelophylax lessonae*, *P. ridibundus*, *P. perezi* and *P. saharicus*, and the kleptons *P.* kl. *esculentus* and *P.* kl. *grafi*, appear to be affected by the anomaly, but possibly other taxa might also be involved in some cases.

No report of anomalies clearly belonging to anomaly P in other groups of frogs traditionally referred to the genus *Rana* but now placed in several subgenera or genera has been published so far. In particularly, this syndrome is unknown in Palearctic brown frogs (genus *Rana* s. str.) and in North American green frogs, now placed in the genus or subgenus *Lithobates*. Anyway, the data available now are enough to state that anomaly P is not species-specific and can touch several taxa in the *Pelophylax* group. Almost everything remains to be done to answer the following questions: (1) are some of the taxa of *Pelophylax* more sensitive than others to the anomaly P, and are some of them immune from it?; (2) more specifically, in the mixed populations involving two or more distinct taxa of *Pelophylax*, does the anomaly P touch them indiscriminately, or not?; (3) does the sensitivity to the anomaly P depend in any way on the special kind of meiosis that occurs in kleptons?

These questions make sense especially as no hard data exist to document the fact that amphibian species of groups other than the genus *Pelophylax* can be touched by the anomaly P, even in the habitats where this anomaly is present in green frogs.

Similar anomalies

Various kinds of anomalies have been described so far in natural populations of many amphibian species. Most of them were found only in isolated individuals. In a rather limited number of cases, evidence has been gathered regarding the rates in populations and/or the causes of anomalies. Two major categories of causes exist for anomalies: genetic and non-genetic. Genetic anomalies due to spontaneous mutations are

usually observed at low (a few percents) or very low (a few per thousands) rates in populations. Non-genetic anomalies may have very diverse causes, and in some cases may occur at very high rates. Some of these epigenetic causes, e. g., pesticides or parasitism, have been elucidated, but in a few cases the causes, although shown or supposed to be non-genetic, are still mysterious and in this respect remind anomaly P. Few of them have been studied as thoroughly as anomaly P, but, for a solution of the latter problem, re-examination of these few cases might prove rewarding, although some of them might later be found to have very different kinds of causes. A review of the cases of mass anomalies so far reported in amphibian populations, which have certainly or probably non-genetic causes and which remind anomaly P in some respect, will be provided elsewhere.

Conclusion

The variability, and hence the unpredictability, of the appearance, frequency, and severity of the anomaly P in a given pond and at a given spot is a serious handicap for the study of its causes. In the world of contemporary research, where obtaining rapidly publishable results is a constraint for obtaining funding for research, this difficulty is a major one for pursuing such studies. Much still remains to be known about anomaly P: its cause, its geographic distribution, exactly which taxa are affected and why, what is the impact of this syndrome on frog populations, etc. This problem attracted the attention mainly of a single biologist, Jean Rostand, in the years 1950–1970, but no studies are apparently under way nowadays, in any laboratory or European country, to elucidate these questions. This is surprising and even shocking, especially in view of the strong interest raised in the recent years by amphibian anomalies in conservation biology. Given the fact that this syndrome involves facts of abnormal cellular multiplication and tissue differentiation and growth, its understanding might throw interesting or important lights on some developmental biology problems. It might also have unexpected implications in conservation biology. More attention should certainly be paid to this unsolved problem by the international scientific community.