3.3. The "anomalie P" and related anomalies

3.3.1. The "anomalie P"

Most of the work concerning the "anomalie P" was carried out by the French scientist JEAN ROSTAND. He had discovered in *Bufo bufo* several cases of polydactyly, some of which seemed to be caused by a recessive gene (ROSTAND 1947a, 1947c, 1950b, 1951b), while other ones seemed due to a dominant gene (ROSTAND 1949a, 1951b). In the hope of finding also a genetic polydactyly in *Rana*, he examined thousands of specimens of this genus, and was thus led to the discovery, at Trévignon in Brittany (western France), of a population of water-frogs exhibiting a very high rate (18%) of polydactyly among adults (ROSTAND 1949b). This polydactyly was quite polymorphic and showed a postero-anterior gradient (ROSTAND 1949b, 1950e, 1951b): in all abnormal specimens the hind limbs were affected by polydactyly (6 to 9 toes), whereas the fore limbs were touched only in some cases, and in particular when the polydactyly in the hind limb was strong (more than 6 toes).

Contrary to the expectation, cross experiments between polydactylous and normal frogs, and also among polydactylous frogs, gave birth only to normal specimens (ROSTAND 1950e), but ROSTAND (1951a, 1951b) was still convinced that a genetic factor, possibly polygenic or with a cytoplasmic transmission, was involved. Examination of the tadpoles of water-frogs in the Trévignon population then established that the rate of anomalies was higher in tadpoles than in adults, and that the anomalies in the tadpoles were much more polymorphic and severe than in adults (ROSTAND 1952b): they included not only polydactyly, but also polymely, brachymely (shortening of leg), various types of bony excrescences and tumours (ROSTAND 1952c, 1955a, 1958a). ROSTAND (1952c) concluded that polydactyly, quite common among adults of this population, was nothing but a discreet and benign manifestation of an anomaly much more common among larvae and which, in its severe forms, caused these to die at metamorphosis. He proposed to call it the "anomalie P" and began to consider the hypothesis of an infectious determinism (ROSTAND 1952c).

In a crucial series of experiments (ROSTAND 1952d), he demonstrated that amputation, in young tadpoles, of the distal part of a hind leg exhibiting the anomaly was followed by regeneration of a normal foot, thus indicating that the teratogenic factor, whatever its nature, had ceased its action at the time of regeneration: such a result, associated with those of the cross experiments, suggested that the anomaly was caused by an extrinsic teratogenic agent having a precocious and temporary action. Many teratogenic factors could *a priori* have been considered, and ROSTAND tried various types of experiments in the hope of reproducing experimentally, in the laboratory, polydactyly and anomalies such as are found in the "anomalie P". However he failed to produce such anomalies by the action of various chemicals (ROSTAND 1950c, 1950d), by rearing eggs and tadpoles in brackish water or in water taken from the ponds where the "anomalie P" did occur (ROSTAND 1952e, 1958a), by the use of various physical stresses such as thermic shocks, long stay at high or low temperature, dehydration, long-continued insolation, etc. (ROSTAND 1950e, 1959), or by inducing overripeness of the eggs (ROSTAND 1951c). By ultraviolet irradiation of larvae just hatched of *Rana temporaria* he obtained some cases of polydactyly, polymely and even brachymely (ROSTAND 1955b, 1958c), but these were morphologically very different from the anomalies observed in the "anomalie P" syndrome, and ROSTAND (1957, 1959) also gave arguments to discard the hypothesis of an action of natural radioactivity. All these observations induced ROSTAND to believe that the "ano-

malie P" was not related to any chemical or physical agent, and led him to favor the hypothesis, first suggested to him in 1949 by CAULLERY (ROSTAND 1952c), of an infectious agent, which might be a teratogenic virus. Since clutches of eggs collected in the Trévignon ponds gave birth only to normal larvae in the laboratory (ROSTAND 1959), it could also be inferred that this teratogenic agent did not step in during the embryogenesis, but between hatching and the appearance of the hind limbs buds.

After various laboratory experiments which failed to bring arguments to support the virus hypothesis (ROSTAND 1952e, 1959, 1960, 1971), a series of experiments realized in the field threw new lights on this problem. While tadpoles reared since hatching in great cages directly immersed in a lake where the "anomalie P" did occur were devoid of any anomaly (ROSTAND, JACQUOT & DARRÉ 1967), tadpoles reared in such cages together with fish (*Tinca, Anguilla*) taken in this lake developed severe anomalies of the "anomalie P" type (ROSTAND & DARRÉ 1967). The sensitive period for the induction of anomalies in these conditions was found to be limited to the first days of the larval life (ROSTAND & DARRÉ 1968). Finally ROSTAND & DARRÉ (1969) succeeded in obtaining abnormal specimens in the laboratory, by feeding tadpoles, just after hatching, with gut contents of fish taken in a lake where the "anomalie P" was common; in such conditions some specimens exhibited even more severe anomalies than those found in the ponds.

It is now clear that the factor inducing the "anomalie P" is present in the digestive tract of fish living in some lakes; that is not to say that it does occur only there and it might quite well prove to exist also in some plants or some aquatic invertebrates of these lakes upon which the fish feed. This factor has still not been isolated but the hypothesis that it might be a teratogenic virus is in agreement with the present data.

This factor seems to be more or less active, in the same population, depending on the year. In the same pond, the anomaly may even disappear for a few years, then appear again. From year to year, in the Trévignon population ROSTAND observed that the frequency of the anomaly could vary between 0 to 80% in tadpoles, and 2.6 to 14.5% in adults (Table 5), while in the Champdieu population the anomaly was found to vary between 0 to 3.1% in young metamorphosed frogs and 0 to 6.3% in adults and subadults (Table 6) and in the Lingé population the frequency of the anomaly in larvae would vary between 14 to 70% (Table 7). Among abnormal larvae, the frequency of the most severe, lethal forms, is also quite different from year to year (Table 5), but is not higher when the frequency of abnormals in the pond is higher; the contrary might even be true (ROSTAND 1959). On the other hand it seems that the most severe forms of the anomaly appear during the years when the total frequency of the abnormals is the highest (ROSTAND 1959).

The frequency of the anomaly in larvae is also different, within the same year, at different dates, and seems to grow when the temperature in the ponds gets higher (ROSTAND & DARRÉ 1969; ROSTAND 1971).

Inside a single pond, the anomaly is not uniformly distributed, but there are some special zones which are far richer than others in abnormal specimens (ROSTAND, JACQUOT & DARRÉ 1967; ROSTAND & DARRÉ 1968, 1969; ROSTAND 1971); from year to year, these zones may shift from one place to another inside the pond and, in response to ecological changes in the ponds which are still not precisely known, they may disappear, sometimes definitively (ROSTAND 1971).

The variability, and hence the impredictability, of the appearance, frequency and severity of the "anomalie P" in a given pond and at a given spot, is, at the point now reached by the study, a serious handicap. In 1970, ROSTAND & DARRÉ (in ROSTAND 1971) tried to

find out whether the teratogenic power of the fish gut contents did resist to ebullition, freezing and filtration but their results were meaningless because, that very year, the anomaly had disappeared from the lake from which the fish came; the same happened to us in 1976, when a whole experimental series of tadpoles, some of which had been fed with

Table 5. Frequency and severity of the "anomalie P" in the Trévignon population of water-frogs, Brittany, western France (after ROSTAND 1952b, 1952e, 1955a, 1955c, 1959, 1960, 1962). × indicate items for which ROSTAND did not publish figures; — indicate that apparently no observation was made.

Year	Tadpoles			Imagos*		Adults	
	n	% "anomalie P"	% severe forms among "anomalie P"	n	% "anomalie P"	n	% "anomalie P"
1949	—	—	—	—	—	256	14.5
1950	—	—	—	—	—	177	13.0
1951	—	—	—	—	—	173	5.2
1952	458	47.2	26.9	153	47.7	117	2.6
1954	537	15.0	45.7	—	—	—	—
1955	435	35.6	47.1	—	—	—	—
1957	×	×	45	—	—	—	—
1958	202	77.2	34	—	—	—	—
1959	×	18	×	—	—	—	—
1960	×	25	×	—	—	—	—
1961	×	80	×	—	—	—	—
1962	×	1	×	—	—	—	—
1963–67	×	0	0	—	—	—	—

* Young frogs just after metamorphosis (see DUBOIS 1978)

Table 6. Frequency of polydactyly (benign form of the "anomalie P") in the Champdieu population of water-frogs, Loire, central France (after ROSTAND 1952b; DUBOIS 1968; DUBOIS & VACHARD, unpublished)

Year (of birth or of observation)	Young frogs, aged one year or less (pooled according to the year of birth)		Adult and subadult frogs, aged more than one year (pooled according to the year of capture)	
	n	% polydactyly	n	% polydactyly
1950	—	—	70	1.4
1951	—	—	960	1.8
1952	—	—	238	4.6
1963	25	0	—	—
1964	603	0	216	0.9
1965	94	0	17	0
1967	653	3.1	15	0
1968	156	2.6	11	0
1969	447	0.5	375	5.6
1970	26	0	—	—
1971	—	—	16	6.3
1974	94	2.1	4	0
1975	428	1.6	—	—
1976	—	—	154	1.3

intact gut contents while others had been given the results of the fragmentation by chromatography of these gut contents, proved equally to have been vain when it appeared that the "anomalie P" had also been absent from the ponds that year (DUBOIS, PAYEN, DARRÉ & TARROUX, unpublished). However, P. DARRÉ told me that in a few other experiments he had found that ebullition had suppressed the teratogenic power of the gut contents; if confirmed, such a result would be a strong argument in favor of the virus hypothesis.

Table 7. Frequency of the "anomalie P" in the Lingé population of water-frogs, Indre, western France (after ROSTAND 1971)

Year	% "anomalie P" among tadpoles
1961	35
1962	60
1964	60
1965	70
1966	14
1970	30

Whatever its causes be, the "anomalie P" is an important phenomenon in the populations of water-frogs where it occurs, since it affects the survival of the frogs (ROSTAND 1962; ROSTAND & DARRÉ 1968). Before metamorphosis the survival of the strongly abnormal larvae does not seem to be affected and artificial or spontaneous prolongation of the larval life allows to keep these specimens alive very long, even over one year and a half (ROSTAND 1959). However, even in protected conditions in captivity, specimens exhibiting the severe forms of the "anomalie P" (including brachymely and various supernumerary bony formations) all die within the first weeks which follow the metamorphosis, merely because of the internal injuries caused by the abnormal bony excrescences in the hind limbs (ROSTAND 1955c). Thus, for pure mechanical reasons, the anomaly in its severe forms is totally lethal at the metamorphosis and only the frogs exhibiting the benign form of the anomaly (simple polydactyly) do survive. Since the frequency of the severe forms often reaches 40 to 50% of the abnormal specimens, which in turn may represent 80 to 85% of the tadpoles in a given population, it is clear that in some cases more than 40% of the tadpoles in a given pond may die at metamorphosis. Such a rate of mortality may strongly affect the populations concerned.

When ROSTAND discovered the anomaly in a Brittany pond, he thought he had found an exceptional phenomenon. However, field and bibliographical research showed that it was not the case:

— Isolated cases of polydactyly, probably referable to the "anomalie P", were found at leat by VIREY (1819) in France, by CHOLODKOVSKY (1896) in the Voronezh region, Russia, by FISCHBERG (in ROSTAND 1951a) in Switzerland, by CAMBAR & HAGET (1949, in ROSTAND 1951a) near Bordeaux, France and by KLAUSEWITZ (1952) in western Germany; in 1977, Dr. H. G. TUNNER sent me the legs of a *Rana "esculenta"* from Austria, exhibiting 8 toes on each hind limb and 5 fingers on each fore limb, anomalies which on morphological grounds clearly belong to the "anomalie P"; in 1978, Dr. J. J. VAN GELDER wrote to me that he had found polydactylous specimens of water-frogs in northern Netherlands and Dr. R. GÜNTHER told me he had seen such specimens in eastern Germany; in Tekirdağ (near Istanbul, Turkey), among 32 *Rana ridibunda* examined in 1969 and 1971, I found an adult male which

had 6 toes on the right hind limb: morphologically the polydactyly was quite similar to the weakest forms of the "anomalie P" observed in Champdieu, but a single case of unilateral polydactyly is not enough to be sure of the occurrence of the anomaly in this region.

— As for massive occurrence of polydactyly among adult and young metamorphosed water-frogs, it has been recorded from Villars-les Dombes, France (BONNET & REY 1937) and Champdieu, France (ROSTAND 1951a, 1952b; DUBOIS 1968; DUBOIS & VACHARD, unpublished), and Dr. L. J. BORKIN told me that in some populations of the region of Minsk (Bielorussia, USSR) up to 5 to 8% of the adult frogs had 6 toes on the hind limbs.

— Finally, occurrence of the various forms of the "anomalie P" in both tadpoles and metamorphosed specimens was reported from Trévignon, France (ROSTAND 1952b, 1952c, 1952e), Lingé, France (ROSTAND 1957, 1958a; ROSTAND, JACQUOT & DARRÉ 1967), Amsterdam, Netherlands (HILLENIUS 1959), Soustons, Léon and Aureilhan ponds in the Landes, France (ROSTAND 1962), Saint-Philbert-de-Grandlieu, France (ROSTAND & DARRÉ 1967, 1968) and Kenitra, Morocco (LAUTIÉ in ROSTAND 1971).

If all the above cases really belong to the "anomalie P", the distribution of this anomaly includes north-western, western, south-western, central and eastern France, Switzerland, eastern and western Germany, Austria, the Netherlands, the USSR, Morocco and possibly Turkey; to this list, ROSTAND (1971), without indicating his sources, adds Greece and Poland. Such a distribution indicates that several forms or species of the *Rana "esculenta"* complex can be touched by the "anomalie P": in France the populations concerned are either mixed *Rana lessonae — Rana "esculenta"*, with both forms exhibiting the anomaly (personal observations in Champdieu), or possibly pure *Rana "esculenta"* populations; in central and eastern Europe, the three forms *Rana lessonae*, *Rana ridibunda* and *Rana "esculenta"* might be involved, while in Greece and in Morocco it must be *Rana ridibunda*, since in these countries the complex seems to be represented only by this form (HOTZ 1974). To my knowledge, no anomaly referable to the "anomalie P" has still been reported in the other species of the *Rana "esculenta"* complex (*Rana perezi* and the forms from Italy and from eastern Asia). It would be interesting to know whether these species are also touched or not, and to map the distribution of the "anomalie P" more precisely than has been done so far.

3.3.2. Related anomalies

Since he had found no abnormal specimens among the other species of Amphibia (*Hyla arborea*, *Bufo bufo*) cohabiting with water-frogs in the ponds where the "anomalie P" was found, ROSTAND (1955a, 1955c) was convinced that the teratogenic factor responsible for the anomaly had a very specific action. However, limited observations suggest that it might not be the case, and that in other species the same factor might have a weaker action than in water-frogs, or a different one. Thus, in *Rana temporaria*, various anomalies (ectrodactyly, syndactyly, etc.) were obtained by rearing larvae in contact with mucus of fish from a pond where the "anomalie P" did occur (SURLÈVE-BAZEILLE, CAMBAR & MAUGET 1969). Also, in Champdieu, quite important numbers of newts (*Triturus helveticus* and *Triturus cristatus*) exhibiting various limb anomalies were collected (DUBOIS & VACHARD in DUBOIS & THIREAU 1972). It is not at all proved whether the above anomalies were really caused by the "anomalie P" factor, but it might be the case (DUBOIS 1974). It should be noted that in some populations of *Rana temporaria*, ROSTAND (1952a, 1955a) has described a non-hereditary ectrodactyly which might also be caused by an extrinsic teratogenic