NEW DATA ON LIMB DUPLICATION IN FROGS UNDER NATURAL CONDITIONS

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Translated from Byulleten' éksperimental'noi biologii i meditsiny Vol. 49
No. 3, pp. 102-105, March, 1960
Original article submitted April 7, 1959

The phenomenon of duplication of the primordial hind limb of the pond frog, unique in the vastness of its scale, has been analyzed in a series of communications [2 5]. Material was collected from three unconnected natural ponds, situated in the foothills of the Zailiiskoe Ala-Tau, over a period of eight years beginning in 1947. A common feature of these ponds was an abundant supply of cold spring water. Abnormalities of development of the frogs caught in the different ponds were largely of one type: They were mainly limited to the region of the right hind limb, but extended in isolated cases to the region of the left limb also [5].

Our efforts to continue the study of this phenomenon have been unsuccessful in recent years on account of extensive improvement work in the area of these ponds. Examination of other ponds, especially those at a higher altitude, gave negative results, since the vast numbers of larvae and young frogs that were caught revealed no anomalies whatsoever.

Subsequently, therefore, we directed our main attention to the irrigated areas of the depression, situated at the same altitude as, or slightly below, the previous ponds. At the end of the summer and in the autumn of 1958, Z. A. Ivanova (laboratory assistant) found large numbers of anomalous late tadpoles and young frogs in two new ponds. These ponds were situated 6 km to the northeast of the main pond where our earlier investigations had been made [2]. Each of the two new ponds was supplied with cold spring water, which enters them abundantly from beneath the south bank, i.e. on the side of the nearest mountain range.

Four catches made in the second half of August and in September gave the following results: in the first, 122 specimens were caught, of which 16 (13.1%) were anomalous; in the second, 369 specimens were caught, of which 30 (8.1%) were anomalous; in the third catch, 34 of 344 specimens (9.8%) showed abnormalities; in the last, 50 specimens were caught, of which 6 (12%) were anomalous.

All the tadpoles with anomalies were greatly retarded in their general development. In this locality metamorphosis of the bulk of the larva of the pond frog is complete at the end of June to the beginning of July. At the catches made in the new ponds during August and September, a considerable part of the material consisted of tadpoles in different stages of development, in addition to young frogs. For instance, of the total number of 86 anomalous specimens which we collected, 57 were tadpoles and only 29 were young frogs of that year. Delay in general development was also shown by discrepancy in the growth of definitive organs and in the tempo of resorption of the larval organs: Growth of the hind limbs took place before resorption of the tail. We specially selected ten anomalous tadpoles (at the stage before appearance of obvious signs of resorption of the tail) and compared them with normally developing tadpoles with the same body and tail weight from the same ponds (see Table).

It can be seen from the Table that at a stage close to the full completion of metamorphosis, the weight of the hind limbs was approximately the same as the figure observed for the anomalous specimens, retarded in development, at an earlier stage. In the tadpoles possessing supernumerary limbs, the duration of proliferation of the main hindlimbs was thus increased, against a background of general delay in metamorphosis when the pond temperature was low (see Figure, a). We have shown earlier that an experimental increase in the duration of proliferation of the hind limbs has a strongly inhibitory action on metamorphosis [1].

Among the new material, seven main variations should be distinguished: The supernumerary limb was completely fused with the main limb as far as the paw - 34 specimens; the supernumerary limb was well differentiated and typically pigmented but was smaller than the main limb, for it was fused with the latter only in the region of the thigh - 18 specimens (solitary discoveries of this type have been described at various times [6]; on the right were three unequally differentiated hind limbs - 8 specimens (see Figure, 2); the supernumerary limb was not fused with the main limb but was attached in the region of the base of the right thigh, orientated in a lateral-cranial direction, well differentiated only in the distal portion and the skin depigmented

Comparative Data of Anomalous and Normally Developing Tadpoles

Conditions and stage of development	Weight (in mg)		
	of the whole larva	of the tail	of both hind limbs
Normal (stage III)	2 480	506	54
With a supplementary right limb weighing on the average 63 mg (stage III)	2 505	513	522
Normal (late stage V)	1 470	21	476

- 10 specimens; deformed supernumerary structures were fused with the proximal part of the thigh of the right hind limb - 11 specimens; the morphogenetic anomalies extended also to the region of the left hind limb - 2 specimens; the right hind limb was completely absent - 3 specimens.

A conspicuous feature was the predominance of cases of symmetrical duplication, as shown by the complete fusion of the ventral surfaces of the main and the supernumerary limbs; under these circumstances the two limbs may be indistinguishable from each other in size and morphological signs. In such cases the thigh and leg were joined together as mirror images, but in the region of the paw full union was not present and fusion was limited to the medial borders, sometimes with reduction of one or two of the medial digits. As a result, a conjoined fan was obtained, spread out in one plane, consisting of 10, 9, 8, 7 or 6 digits.

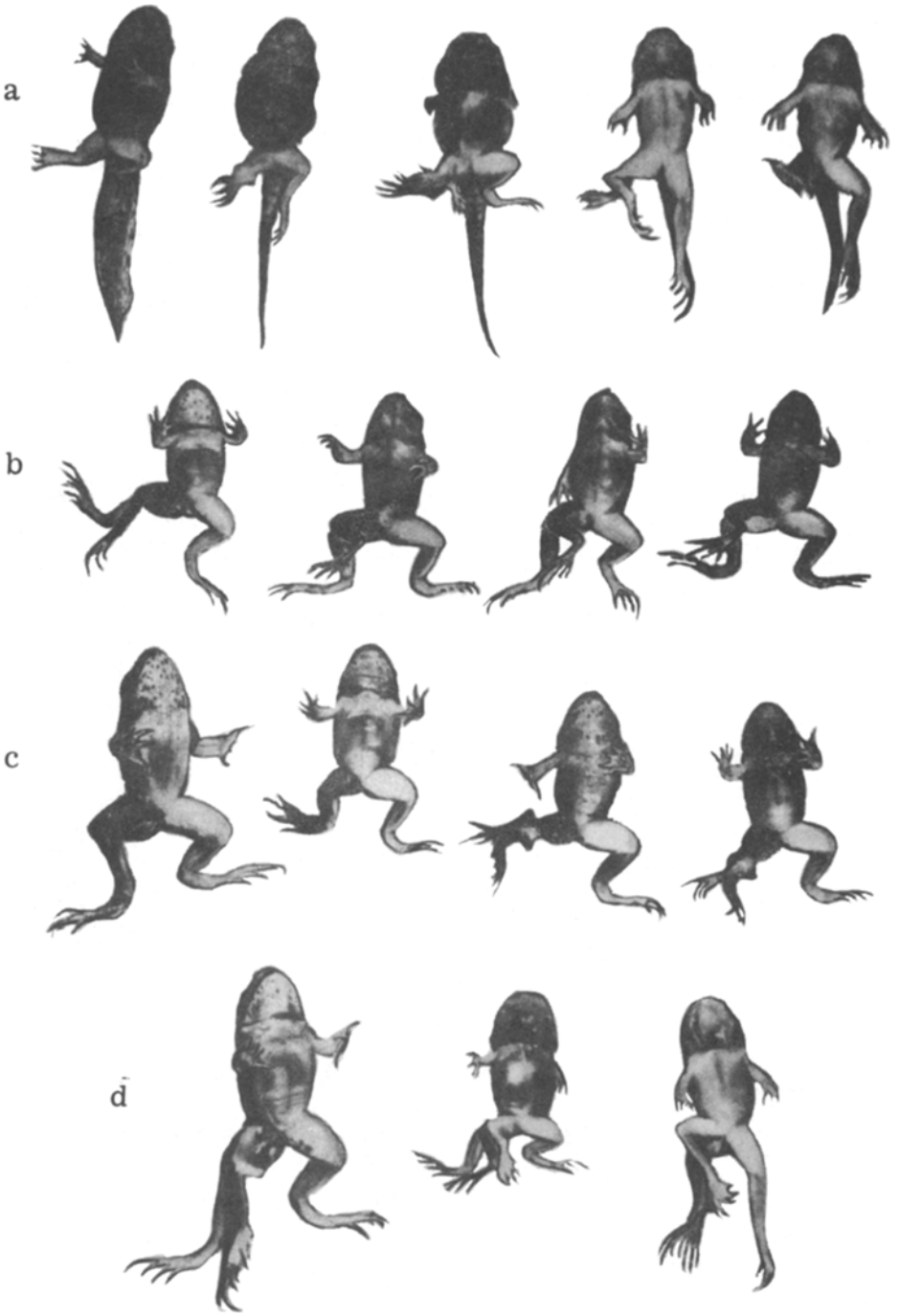
Such conjoined limbs had a common skin covering, highly pigmented over its whole extent according to the dorsal type (see Figure, c).

More than half the anomalous specimens with this mirror-image arrangement had a conjoined limb which was shortened by comparison with the single left limb. The shortening was due to the symmetrical transformation of the bones of the leg and the distal part of the thigh into spicular lateral processes in the two conjoined limbs. Altogether there were 22 specimens with spicular transformation of the middle part of the conjoined limb, or 25.6% of the total number of anomalies found (see Figure, c). Among the 304 anomalous specimens previously collected, 47 (15.4%) had this spicular deformity of the leg, predominantly affecting the right main limb. The supernumerary limb was often severely maldeveloped and very characteristically fused to the tissue of the main limb, so that one normal digit was incorporated as a sixth component of the paw of the main limb [4]. In the new material (see Figure, c), in 19 specimens (22.1% of the total number of anomalies) complete symmetry was observed, with an analogous spicular transformation of the leg of the supernumerary and main limbs (compared with 1.3% of the total number of 304 anomalies collected earlier). Only in three specimens was the spicular structure limited to the main limb, but in these cases too, the presence of a sixth digit in the paw of the main limb was not observed.

Another significant feature of the conjoined limbs in the new material was the normal orientation of the distal segments in the majority of anomalous specimens, whereas in 47 old specimens, possessing spicular structures, rotation of the paw through 180° was observed [4]. Rostans [7] observed in the green frog closely similar morphological deformities in the region of the leg and thigh and called them P anomalies. The main signs of these abnormalities were rotation of the paw in a cranial direction, transformation of the leg bone and part of the femur into a common, massive cone, severe shortening of the limb and, in several cases, the presence of a varying number of supernumerary digits in the paw. In such specimens a bilaterally symmetrical deformity of the right and left limbs was always observed. Comparison of our material with the anomalies shown in the numerous photographs accompanying the paper by Rostans [7] permits several signs of agreement to be recognized, but the morphological pictures are not completely identical.

In some cases, when the number of supernumerary limbs on the right side was two or three, we observed a gradation in their degree of development (see Figure, d); the least differentiated limbs were without pigment. An analogous phenomenon, although less well marked, may be seen in the photographs of some of the anomalous specimens (see Figures 27, 28 and 29 in the monograph by Rostans [7]), but a clear bilateral symmetry is observed here.

In 18 specimens the supernumerary limb was joined to the main limb only in the region of the thigh. In late tadpoles and newly developed young frogs such limbs were well differentiated, but were much smaller in size than the main limb (see Figure, b). At early stages of larval development the dimensions of the small supernumerary limbs were very little different from those of the main limbs. In the later period the supernumerary limb gradually falls behind in growth. This lag in growth is the more considerable the smaller the degree of fusion of the supernumerary limb with the main. For instance, in ten cases in which the supernumerary limb was in contact with the main limb only in the region of the pelvis or base of the thigh, only the distal parts of the supernumerary limbs were well formed, and in 11 cases shapeless fibro-osseous outgrowths developed, covered with depigmented skin. The trophic contact between the two limbs, restricted to the region of the thigh, ensured



Tadpoles and young frogs with supernumerary limbs. a) 5 anomalous tadpoles in various stages with massive hind limbs and delayed resorption of the tail; b) 4 young frogs with supernumerary limbs (fusion of the ventral surfaces restricted to the region of the thigh); c) complete fusion in the region of the thigh and leg (on the left – without deformity, on the right – with symmetrical deformity of the leg); d) the presence of a second supernumerary depigmented limb, situated beneath two other variously fused right limbs.

the harmonious development of the supernumerary limb, but with a lag in its growth that increased progressively with time (18 cases).

Close trophic contact between the two limbs, joined together in the region of the thigh and leg (34 cases), brought about growth of the supernumerary limb to roughly the same extent as growth of the main limb.

A common feature of the new material on anomalies and that which we collected and studied previously in the uniform character of the disturbance of morphogenesis in the region of the primordial right hind limb, which is found in variants from the local extinction of morphogenesis to the formation of several supernumerary limbs. The principle of duplication of the material of the anlage was revealed most clearly among the anomalous specimens of the new material. The level of morphological differentiation and the growth of supernumerary structures are directly dependent on the degree of anatomical fusion and trophic contact of the supernumerary and main limbs.

SUMMARY

A total of 86 new specimens of late tadpoles and young frogs (Rana ridibunda) with supernumerary right posterior extremities were caught in ponds. The growth and the morphological differentiation of the supernumery extremities depended directly on their anatomical connection of the supernumerary extremities with a spicular deformity of the shin was of a relatively frequent occurrence. Peculiar gradation was noted in cases of development of two and three supernumerary extremities.

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