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What are the key advantages and disadvantages of urodele species compared to anurans as a model system for experimental analysis of early development?

PIETER D. NIEUWKOOP*

Hubrecht Laboratory, Utrecht, The Netherlands

As an experimental morphologist I shall restrict myself mainly to the usefulness of the various urodele and anuran species for the morphogenetic analysis of embryonic development, leaving the discussion of the biochemical and genetic approaches to other specialists.

First of all we must ask ourselves, "What are the main requirements for the adequate experimental animal"? These turn out to be many: e.g., good local availability, easy adaptation to laboratory conditions, proper length of breeding season, relatively easy manipulation of eggs and embryos, adequate speed of embryonic development, not too long a generation time, proper histological differentiation of embryo and larva, etc.

It is the morphogenetic analysis which has led to the delimitation of successive steps in the build-up of the rapidly increasing complexity of the epigenetically developing organism from egg to larva and adult. First of all one has to know *when* and *where* inside the embryo certain interactions occur during its epigenetic development before one can successfully start the biochemical and genetic characterization of the factors and genes involved.

A first requirement for successful experimental analysis is a thorough knowledge of normal development, external as well as internal. For the knowledge of external development a detailed Normal Table of the species in question is essential, while for a good understanding of the internal development a proper knowledge of its anatomy and histology are required. The latter is evidently becoming a more and more uncertain factor, as can be judged from the modern literature, which has led in several cases to incorrect and even unjustified conclusions.

It is perfectly evident that we cannot speak of advantages and disadvantages of *the* urodeles and *the* anurans as separate groups. We must actually compare the most suitable representatives of the different amphibian groups.

First of all we cannot ignore the historical development in this branch of science, due to the accumulation of experimental data on particular species, species which were probably initially chosen on the basis of local availability and personal preference. This has led to the following development. Apart from some initial experiments by W. Roux and others on anuran embryos, the thorough analysis of early vertebrate development was actually started at the beginning of this century in the German school of H. Spemann (see Spemann, 1921 and Spemann and Mangold, 1924) with Mangold (see Mangold and Spemann, 1927), Holtfreter (see Holtfreter, 1933) and others using urodeles, notably *Triton* species, now classified under the genus *Triturus*. The American school of R.G. Harrison (see Harrison, 1918) also used urodeles, but *Amblystoma* (a typing error led to the taxonomically correct, but senseless name *Ambystoma*) species, notably *Ambystoma punctatum*. During the thirties, the Mexican axolotl, *Ambystoma mexicanum* became widely introduced in Europe by Holtfreter (1933) and in the USA by R.R. Humphrey and G. Fankhauser (1946). V.C. Twitty (1942) added the Californian urodele species, *Triturus torosus*, now classified under the name *Taricha torosus*, while Okada and coworkers (see Okada and Hama, 1945 and Okada and Ichikawa, 1947) introduced the Japanese urodele species, *Triturus pyrrhogaster*, now classified as *Cynops pyrrhogaster*. *Pleurodeles waltl* has been extensively used by Boucaut *et al.* (1979) and Duprat *et al.* (1982).

Among the urodele species, *Triturus taeniatus, Tr. cristatus, Tr. alpestris,* and *Tr. palmatus* and likewise *Ambystoma mexicanum* found general use in European embryological research during the

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first half of this century (see Nieuwkoop, 1947). Whereas the different *Triturus* species have a reasonably long breeding season of several months during the spring, artificial breeding could only be induced in the axolotl, *Ambystoma mexicanum*, in the laboratory during a period of 5 to 6 months each year by means of a temperature drop of ±10%C in the aquarium. Although the natural breeding season of *Ambystoma punctatum* in early spring is very short, its geographical distribution over quite a range in altitudes, where spring starts at different times, allowed a reasonable experimental season due to a well-organized shipment of freshly laid eggs. The same holds more or less for the American species, *Ambystoma tigrinum, A. opacum* and *A. jeffersonianum*.

Among the anurans, *Rana, Bufo* and *Bombina* species were initially used in Europe, and *Rana pipiens* and other *Rana* species in the USA. The introduction of the South African clawed toad, *Xenopus laevis*, in many medical and biological institutes during the fifties, initially for use in human pregnancy tests, led, however, to the nearly complete dominance of that species in developmental biological research.

Nearly all *Rana* and *Bufo* species have a very short breeding season, which also holds for the treefrogs, *Hyla regilla* and *H. arborea*. These genera are, moreover, rather unsuitable laboratory animals. On the other hand, *Xenopus laevis* can be reared very easily under laboratory conditions, while breeding can be initiated experimentally by gonadal hormone injection throughout the *entire* year. It is certainly the last fact that led to its preference over all other anuran species and also over the various urodele species, particularly for biochemical and for genetic research.

The development described above has led to the rather unfortunate situation that, although developmental biological research started on and concentrated almost exclusively on urodele species till the middle of this century, the following decades show a nearly complete shift towards anuran development, notably to *Xenopus laevis*. Now it is actually becoming necessary to present a survey of the actual advantages and disadvantages of the use of urodele species in comparison with *Xenopus laevis*.

The European *Triturus* species have the great advantage of different species-specific characteristics which are very suitable for identifying host/graft boundaries in heterospecific chimeras, e.g., due to color and abundance of embryonic pigment, cell size and specific organ formation. These criteria can be used as very good genetic markers. The *Triturus* species show a very satisfactory embryonic and larval histological differentiation. There are, moreover, no incompatibility reactions during embryonic or larval development. The various *Triturus* species have eggs of nearly the same size and show nearly the same speed of development. The availability of eggs during only a part of the year is a certain disadvantage, particularly since one often needs eggs at the same stage of development. *Triturus* eggs are deposited one by one. This does not hold for *Ambystoma* species, which deposit their eggs in large quantities and with little or no variation in age.

Ambystoma eggs are slightly larger than *Triturus* eggs, but a heterospecific combination of both genera is perfectly satisfactory, the speed of development being hardly different. The geneticists complain that the urodeles, in particular *Ambystoma* species, contain a much larger amount of DNA per cell, which should make the preparation of DNA libraries much more laborious.

One of the great advantages of urodele embryos for experimental analysis is the single-layered nature of early developmental stages, due to which the gastrulation process can easily be followed from the outside. The same holds for the subsequent neurulation process (see Vogt, 1929). This strongly facilitates accurate excision and transplantation of particular embryonic anlagen. On the other hand the relatively low speed of development has certain disadvantages, since decapsulated embryos have to be kept under proper sterility conditions for at least one week.

All urodele species require one to two years to reach sexual maturity, which certainly hampers genetic analysis. The urodeles have however the *great* advantage that there is a very thorough documentation of their morphogenetic development, executed during the first half of this century, which forms a very sound and indispensable base for biochemical and genetic analysis.

As we have seen, *Xenopus laevis* occupies a very special position among the anuran Amphibia, essentially because it is a very suitable laboratory animal, which can easily be kept and bred under laboratory conditions, but particularly because breeding can be introduced by hormone injection through the *entire* year. Using the method of stripping injected females and fertilizing the eggs artificially by maceration of an excised testis, large numbers of simultaneously fertilized eggs can be obtained, which is ideal for biochemical work. However, there are some serious limitations connected with the use of *Xenopus* eggs. The main restriction relates to the double-layered nature of the totipotent animal moiety of the *Xenopus* blastula/gastrula and neurula stages (Nieuwkoop and Florschütz, 1950). In contrast with other anuran species such as *Rana* and *Bufo*, where the presumptive mesoderm is at least partially situated at the outer surface of the embryo, mesoderm formation in *Xenopus* is entirely restricted to the sensorial layer of the equatorial region of the embryo, the outer epithelial layer only contributing to the archenteron endoderm. The situation of a *purely internal marginal zone* has rather far-reaching consequences for the gastrulation process. It leads to very precocious and largely independent involution of the mesoderm, causing the initiation of vertical neural induction in the overlying ectoderm before any invagination of the endodermal archenteron has actually taken place (see Keller, 1976; and Nieuwkoop and Koster, 1995). Gastrulation cannot therefore be followed properly from the outside. The neural anlage is also nearly exclusively formed out of the inner sensorial layer of the ectoderm, only the dorso-median region of the outer epithelial layer forming the future endothelium of the neural tube.

The rather small *Xenopus* embryo (±1.5 mm) is far less suitable for experimental intervention than, e.g., the axolotl embryo (±2.5 mm), the more since the presumptive marginal zone as well as the presumptive neural anlage occupy only narrow strips at the equatorial and supra-equatorial regions of the embryo respectively. Extirpation of presumptive organ anlagen, particularly in the animal-vegetal direction is therefore difficult and rather inaccurate.

There is only one other *Xenopus* species, *Xenopus tropicalis*, with which heterospecific recombination can be undertaken. Whereas *Xenopus laevis* is a subtropical species, the much smaller *Xenopus tropicalis* is a tropical species, which develops properly at a higher temperature. The eggs are moreover much smaller, and this more delicate animal is less suitable as a laboratory animal. Sexual maturity is reached in *Xenopus laevis* in about one year, which is not much earlier than in the urodele species.

At present *Xenopus laevis* has the great advantage of being nearly exclusively used for biochemical and genetic analysis during the last decades, so that it has gradually acquired a thorough lead as experimental animal.

We must admit, however, that its morphogenetic analysis lags seriously behind that of the urodeles and that its normal development is far less well studied than that of *Triturus* and *Ambystoma*. Happily, attention has recently been directed to the present discrepancies between the use of the different urodele species and *Xenopus laevis*.

For a more rapid succession of generations, so important for genetic research, we must turn to, e.g., the small tropical zebrafish, *Danio regio*, or to the mouse. Yet these vertebrates show other limiting factors for developmental biological research: for example, the nearly unknown morphogenetic development of fish embryos in general and of the zebrafish in particular, as well as the early implantation of mammalian embryos. Cultivation of the latter outside the uterus is only possible for a short period. Conversely, much information has been gathered during the last decades on the genome of the mouse, but in the zebrafish genetic analysis is still in its infancy.

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