

The embryonic development of *Pleurodeles waltl* (Michah) Reappraisal of the Gallien-Durocher stage series

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Among the Urodelan amphibians that were chosen for study by developmental biologists during the second half of the present century, the Spanish newt (*Pleurodeles waltl* Michahelles) was and still is widely used in European countries and especially in France. Concomitantly with other European newts, it had already been selected by Vogt (1929) to establish the map of presumptive areas in the Urodelan gastrula: compared to the *Triturus* species, the *Pleurodeles waltl* offers a great advantage by providing hundreds of eggs in one and the same spawning, an abundance shared by the Mexican Axolotl, whose eggs and embryos are slightly bigger.

A decisive step forward was taken when laboratory facilities were established to breed *Pleurodeles*. The moving spirit behind this task was Professor Louis Gallien (University of Paris) who published in 1952 a short article describing the mating behavior of adult *Pleurodeles* kept in aquaria, the collection of eggs, the rearing of larvae and young post-metamorphic animals. The latter were prevented from leaving the water, which enable Gallien to acquire a population of salamanders that were constantly maintained in running water and could spawn, not only in winter and spring, like the wild populations of Portugal and Spain, but also in autumn. A selection of such animals probably occurred at the beginning, because young adult wild animals obviously suffer and eventually die when they are constantly maintained in water. Even young metamorphosed animals, when obtained from wild parents, are not quite able to adapt to a permanently aquatic existence. Gallien's original population of Spanish newts in 1948 was obtained from a few adults kept in the Museum National d'Histoire Naturelle in Paris. Later on, crosses with wild animals from Portugal were performed to avoid the deleterious effects of perpetuated inbreeding.

In the early fifties, standard developmental stages had already been described for several species of frogs, toads and salamanders (*Ambystoma*, *Triturus*). It was necessary to obtain a similar stage series for *Pleurodeles*, which Gallien had definitely chosen as a developmental model at that time, when his investigations on frog sexual differentiation shifted to the Urodeles, in parallel to Humprey's work on *Ambystoma*. I was still a student in October 1950 and I had to work on a biological research topic for one year. Gallien allowed me a choice of two topics. The first was an investigation of the regenerative power of limbs in post-metamorphic *Xenopus*, which had not yet been described. The second was the time-table of *Pleurodeles* embryonic development. I preferred the first alternative because it was an experimental topic and, moreover, at that time, there were no temperature regulated incubators (18° or 20°C, 25°C) in the newly established laboratory: *Pleurodeles* eggs still had to be kept in more or less well insulated glass containers and heated with electric lamps, a situation which was not satisfactory for precise time-staging.

Only a few years later came another student, Micheline Durocher, from the Ecole Normale Supérieure de Fontenay-aux-Roses, where Gallien used to give regular lectures. For several months, she devoted herself conscientiously to the study of *Pleurodeles* development. She found evidence for a slight variability in the duration of stages from one spawning to another and she calculated the limits. She accurately drew the various embryonic

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0214-6282/95/\$03.00

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Printed in Spain

stages and described their precise characteristics. A comparison of developmental stages in *P. waltli* and several other salamander species was established, too. Actually, the stages for *P. waltli* were numbered according to those for *Triturus taeniatus* (*Molge vulgaris*) that Glaesner had published as early as 1925. In that case too, the stages were described until the end of metamorphosis. At $18^{\circ}\pm 0.5^{\circ}\text{C}$, development seemed to proceed at a similar rate in both species, at least for the first week. Durocher still compared the development of *Pleurodeles* embryos at two temperatures, 18°C and 25°C : in the latter case, some abnormalities were seen to occur, and the results were consequently not published.

The bulk of the data describing the developmental table at $18^{\circ}\pm 0.5^{\circ}\text{C}$ and the comparisons with several other species, appeared in 1957 in the *Bulletin Biologique de la France et de la Belgique*, a well-known French-Belgian quarterly journal. This reference has been cited ever since by all researchers working on *Pleurodeles*, probably several hundred citations in all.

The progressively increasing difficulty in obtaining the original article from a foreign library could only be overcome by personal exchanges of copies of old reprints. Add to that some limitations or inaccuracies in the original description, and the moment was ripe for a new issue of the developmental table.

We are thus indebted to Professor Jean-Claude Boucaut, one of Gallien's former students, and to his own collaborators for having collected new illustrations from their research work and/or having checked the successive developmental stages of *Pleurodeles waltli*. The authors are particularly interested in the gastrulation stages and their presumptive areas. *Pleurodeles* remains a useful model to study Urodele embryogenesis as compared to *Xenopus*. Gastrulation in Urodeles occurs much more slowly than in *Xenopus* and the behavior of cell sheets is different too. Thus, for example, lithium treatment of early cleavage stages resulted in a peculiar form of dorsalization in *Pleurodeles gastrulae* (Shi *et al.*, 1990). New investigations on the fate maps were recently initiated by using microinjection of fluorescent dyes into separate gastrula cells (Delarue *et al.*, 1992). These data provided the authors with photographic illustration which is put to relevant use in the revised developmental stage series.

When Gallien selected *Pleurodeles* embryos and larvae as his favorite model, he was working on experimental sex reversal in Amphibians and its genetical aspects. But the PhD theses written under his supervision were also devoted to other topics, many and various: morphogenetic effects of experimental heteroploidy and lithium treatment of the early gastrula, effects of Spemann organizer grafts on later organogenesis, origin of neural crest cells, chromosomal abnormalities as a consequence of egg irradiation, nuclear transplantation, etc... *Pleurodeles* was then introduced into several developmental biology laboratories in France in the early sixties, when Gallien's collaborators themselves created autonomous teams, which in turn led to diversification of research topics.

At present, the use of molecular techniques unfortunately appears to be more difficult in Urodele Amphibians than in *Xenopus*. This should not be a constitutive defect, provided that convenient molecular probes be prepared. I am still confident that *Pleurodeles* and *Ambystoma* will remain useful models to investigate basic problems that have most probably received specific, and necessarily general, solutions in the evolutionarily specialized *Xenopus* embryo.

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