

# **Adaptations of the amphibian egg and hatchling for terrestrial nesting in the Wenxian newt (*Tylototriton wenxianensis*)**

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Reducing dependence on aquatic habitats for reproduction is a hallmark in vertebrate evolution. Salamanders of the family Salamandridae typically produce aquatic eggs that develop into a free living aquatic larva. Few salamandrid species (genera *Tylototriton* and *Echinotriton*) have adapted to egg laying on land with subsequent aquatic larval development.

We here study the mechanisms that allow successful development of salamandrid eggs on land using the Chinese salamandrid *Tylototriton wenxianensis*. We identified the mechanisms enabling 1) the eggs to develop on land without dehydration 2) hatchlings of *T. wenxianensis* to successfully reach surface water for further development. Water loss and gain rates were compared between eggs of *T. wenxianensis* with those of aquatically reproducing salamanders and agar balls. The contribution of the egg fluid and egg outer membrane to water homeostasis was assessed by replacing egg fluid with water or by removing the outer egg layer. The contribution of egg fluid proteins was assessed using SDS PAGE. The movement of hatchlings towards different potential stimuli was studied in an experimental arena and musculature was examined using histology.

Eggs of *T. wenxianensis* showed high resistance to fluid loss through a combination of the presence of high molecular glycoproteins and retaining a globular shape. Hatching was limited to low light conditions and hatchlings, put on a horizontal surface, actively jumped to a light source using densely organised epaxial muscles, covering distances up to 2.8 m per hour. These adaptations are likely to maximize chances of reproduction success of terrestrial nesting.