Evolution of Viviparity in Salamanders (Amphibia, Caudata) (A22851)

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Abstract (120-150 words)

Reproductive modes in salamanders (Amphibia, Caudata) are highly diverse. Viviparity, for instance, implies the retention of the developing embryos inside the females until the end of the gestation, at which point they deliver fully developed terrestrial juveniles. From an ecological point of view, the evolution of viviparity is highly significant, since it implies the semi-independence of water for an amphibian. Nevertheless, viviparity is not very common among salamander. It has independently evolved only in a few species, all included within the family Salamandridae. Furthermore, the characteristics of viviparous strategies are different among species, although they all share some commonalities. The detailed study of these particular and common features in a phylogenetic context will reveal the genetic, physiological, developmental, morphological, and historic factors that have triggered the evolution of this peculiar mode of reproduction in only one lineage among all the species of salamanders.

Key words:

Development, Embryology, Evolution, Larvae, Life history, Metamorphosis, Phylogeny, Reproduction.

Key features:

- Viviparity entails the complete development of the progeny within the mother's genital tract, together with the maternal provisioning of nutrients to the embryos.
- Since development occurs within the females, viviparity in amphibians implies that the aquatic phase of the standard amphibian life cycle is obviated in viviparous species.
- Viviparity has independently evolved only in a few species of salamanders.

Introduction

Amphibians are generally characterized by a biphasic life cycle that involves (i) an adult terrestrial phase, (ii) the mating of adults in water, (iii) the delivery of eggs in water by the female, (iv) the external fertilization of eggs by the male, (v) the development of embryos within the egg membranes, (vi) the hatching of developed embryos in water (i.e., larvae), (vii) a free living stage as aquatic larvae, and (viii) the metamorphosis of aquatic larvae into terrestrial juveniles, which starts the terrestrial phase of the life cycle (Oldham, 2001).

However, this standard biphasic life cycle, also known as oviparity, has been repeatedly modified during amphibian evolution. There are currently 6888 described species of amphibians belonging to three orders (AmphibaWeb, October 2011): Gymnophiona (caecilians or limbless amphibians with 188 species) (Milner, 2001a), Anura (toads and frogs, with 6086 species) (Dundee, 2001), and Caudata (salamanders, with 614 species) (Milner, 2001b). Many species of caecilians, frogs, toads, and salamanders have developed remarkable modifications of this life cycle, including different mating strategies, parental care behaviours, and modes of reproduction. [See also: DOI: 10.1038/npg.els.0001536](https://doi.org/10.1038/npg.els.0001536); [DOI: 10.1038/npg.els.0001539](https://doi.org/10.1038/npg.els.0001539); [DOI: 10.1038/npg.els.0001537](https://doi.org/10.1038/npg.els.0001537); [DOI: 10.1038/npg.els.0001538](https://doi.org/10.1038/npg.els.0001538).

Viviparity (or live-bearing) involves the retention of the developing eggs within the females until the end of the gestation, at which point they deliver fully developed terrestrial juveniles. With this mode of reproduction, the different phases of development all occur within the mother's genital tract. Viviparity has independently evolved in most vertebrate groups, except cyclostomes (hagfishes and lampreys) (Janvier, 2001) and birds (Cunningham, 2002). Indeed, this mode can characterize entire lineages such as within mammals (Harrison, 2001). Similarly, viviparity has independently evolved several times within the three orders of amphibians, although its prevalence and occurrence varies among lineages (Wake, 1982). For example, only a few species of anurans are known to be viviparous (approximately 6 out of the more than 6000 known species), while more than half of the caecilians are thought to be livebearers. Interestingly, within salamanders, viviparity only occurs in a few species within the family Salamandridae. [See also: DOI: 10.1038/npg.els.0001532](https://doi.org/10.1038/npg.els.0001532); [DOI: 10.1038/npg.els.0001548](https://doi.org/10.1038/npg.els.0001548); [DOI: 10.1038/npg.els.0001855](https://doi.org/10.1038/npg.els.0001855).

Salamanders (614 currently recognized species) are grouped in ten families. Although still a matter of some debate, the phylogenetic relationships between families are relatively well established (Zhang and Wake, 2009; Pyron and Wiens, 2011) (Figure 1). Clade A, often designated as the suborder Salamandroidea, is the most speciose clade and includes

the seven families of salamanders with internal fertilization (see below). The family Salamandridae is included in this clade. <Figure 1 near here>

Reproductive modes in salamanders

Modes of reproduction in salamanders are rather diverse. For instance, some species of salamanders never complete metamorphosis. In a phenomenon known as paedomorphosis, individuals retain a larval phenotype. They live and reach sexual maturity in the water (Gould, 1977; Horder, 2006). Paedomorphic species obviate the terrestrial phase of the standard biphasic life cycle. This is the observed condition in the well-known axolotl (*Ambystoma mexicanum*). The occurrence of paedomorphosis in some species is driven by the environmental conditions (facultative paedomorphosis), while in some others like the axolotl the strategy is fixed (obligate paedomorphosis) (Denöel *et al.*, 2005). [See also: DOI: 10.1038/npg.els.0004180.](https://doi.org/10.1038/npg.els.0004180)

On the other side of the spectrum, we find species in which the aquatic phase is eliminated. This is the case of viviparous species and direct developers, although this semi-independence from water is achieved by different means in these two cases. Viviparous females retain the developing embryos inside their genital tract throughout development, while in direct developers, females lay the newly fertilized eggs under logs or underground. The embryos develop within the eggs membranes until hatching. Hatchlings are small but fully formed terrestrial juveniles. The aquatic larval phase is, thus, also obviated. Direct development occurs in all of the species of the family Plethodontidae (Fig. 1), which is, by far, the largest family of salamanders, containing about two-thirds of all known species.

An important feature that has permitted the evolution of viviparity and direct development is internal fertilization. In species with internal fertilization, male sperms are packed in gelatinous sacs called spermatophores. During courtship and mating, males deposit one spermatophore on the ground and the female picks it up with her cloacal lips, storing the sperm in a specialized area of her cloaca called the spermatheca (Sever and Brizzi, 1998). Fertilization of eggs need not occur concomitantly with mating: females may store the sperm in the spermatheca (duration is a species-specific trait). The evolution of internal fertilization in salamanders has been a key element for the evolution of these two highly derived reproductive modes.

Viviparity in salamanders

Viviparity entails the complete development of the progeny within the mother's genital tract, from metamorphosis to until they are delivered as small terrestrial juveniles. Furthermore, in viviparous species there is also a maternal provisioning of nutrients to the developing embryos, that is, matrotrophy. These two traits generalize viviparity as a reproductive mode, although we have to bear in mind that under the umbrella of this definition there is a diverse array of strategies required to achieve viviparity that involve different biochemical, cellular, physiological, developmental, behavioural, and evolutionary mechanisms and processes.

In salamanders, viviparity occurs in eleven species, all within the family Salamandridae. The family Salamandridae contains 86 species (AmphibaWeb, October 2011), grouped into two main clades (Figure 2) (Weisrock *et al.*, 2006; Zhang *et al.*, 2008). The first clade includes 71 species of newts, characterized by a high dependence on water. All these species are oviparous, that is, they follow the standard biphasic amphibian life cycle of having aquatic eggs and a free-living larval phase. The second clade includes a group of species of terrestrial or 'true' salamanders. It is within this clade that viviparity has independently evolved several times, specifically within the genera *Salamandra* and *Lyciasalamandra*. <Figure 2 near here>

The genus *Salamandra* contains six species, which are considered either ovoviviparous or viviparous. The two species of Alpine salamanders, *Salamandra lanzai* and *S. atra*, are strictly viviparous, while *S. corsica*, *S. infraimmaculata*, *S. algira* and *S. salamandra* are referred as ovoviviparous. Ovoviviparity is defined as the retention of the progeny within the uterus during the first phase of development, followed by an aquatic larval stage. This mode is considered the ancestral reproductive mode in this genus. Ovoviviparous females ovulate and fertilize a large number of eggs (40 to 100). Embryos develop within the egg membranes in the two uteri and feed exclusively on the nutrients supplied in the egg yolk (i.e., lecithotrophy). After this first lecithotrophic phase, which may last from three to twelve months depending on environmental conditions, females will deliver aquatic larvae in water. These larvae start actively feeding shortly after being released. The aquatic larval phase may last from three months to more than two years depending, again, on environmental conditions, before larvae metamorphose into terrestrial juveniles (Joly, 1994).

This reproductive pattern is modified in the fire salamander, *Salamandra salamandra*. This species is of special interest as it represents one of the few examples within vertebrates to display intraspecific variability in reproductive modes. *S. salamandra* is widely

distributed in Eurasia. Morphological patterns and life history traits are extremely variable in this species. The highest levels of variability are found in the Iberian Peninsula, where up to ten subspecies are currently recognized (Buckley and Alcobendas, 2002). Subspecies of *S. salamandra* have been traditionally categorized based on coloration patterns and morphometric characteristics. Besides the morphological variability, one of the most striking features in *S. salamandra* is the polymorphism in reproduction modes. *Salamandra salamandra* females are commonly ovoviviparous, releasing approximately 20 to 70 small larvae directly into ponds or streams. However, this common pattern of reproduction is modified in the Northern Iberian populations of fire salamanders. The subspecies *S. s. bernardezi* and some populations of the subspecies *S. s. fastuosa* are viviparous (Greven and Guex, 1994; García-París *et al.*, 2003). Viviparity in these populations is characterized, first, by the incomplete fertilization and development of eggs. As opposed to the ovoviviparous populations, not all the ovulated eggs of viviparous females are fertilized. Furthermore, some of the fertilized eggs arrest during early development. As a result, although the number of eggs ovulated are roughly the same in ovoviviparous and viviparous females, fewer embryos develop within the viviparous females. In both cases, embryos first go through a lecithotrophic phase of development, nourishing exclusively on the egg yolk. However, in embryos from viviparous females, this lecithotrophic phase is accelerated and the yolk is rapidly consumed. After exhausting the nutrient reserves, the embryos hatch within the uterus, during early developmental stages, which allows them to move freely within the uterus. Interestingly, hatched embryos present a very peculiar morphology. While most of their organs and body parts, such as limbs, are not fully developed, all of the structures related to feeding (e.g., the opening of the mouth or the digestive tract) are developed and functional. All these structures appear much later during development in ovoviviparous embryos. This constitutes an example of heterochrony, that is, the change in the timing of some events during development compared to the ancestral developmental sequence of the group, in this case, ovoviviparity (Horder, 2006; Buckley *et al.*, 2007). The heterochronic pattern of development enables the embryos to feed actively on the unfertilized and abortive eggs (i.e., oophagy). They also feed on other developing siblings (i.e., adelphophagy) (Dopazo and Alberch, 1994; Dopazo and Korenblum, 2000; Buckley *et al.*, 2007). This intrauterine cannibalistic behaviour in *S. salamandra* is exclusive to this species among salamanders. The additional nutrients provided from oophagy and the intrauterine cannibalism on siblings permits the developing embryos to complete their development within the maternal uteri. As a result of this peculiar reproduction mode, females give birth

to a few (1-15) fully metamorphosed, terrestrial juveniles in just three months of gestation. This is approximately the same amount of time required for ovoviviparous females to lay aquatic larvae on water. [See also: DOI: 10.1038/npg.els.0004180](https://doi.org/10.1038/npg.els.0004180).

Viviparity in *S. salamandra* occurs in populations within a continuous range of distribution of the species. Viviparous and ovoviviparous individuals are in contact and can mate and reproduce, providing an unusual and exceptional natural scenario for studying the evolution of reproductive strategies within the group from a population perspective (García-París *et al.*, 2003; Buckley *et al.*, 2007; Buckley *et al.*, 2009). Furthermore, the occurrence of viviparity has recently been reported in some insular populations of the subspecies *S. s. gallaica* (Velo-Antón *et al.*, 2007). The evolutionary origin of these populations and, hence, of viviparity is different from the case described above for *S. s. bernardezi* and *S. s. fastuosa*, providing a second natural setting to study the evolution of viviparity in *S. salamandra*. Little is known, however, about the physiological and developmental characteristics of viviparity in this insular group, although it is likely related to the absence of water points in the islands (Velo-Antón *et al.*, 2007). The occurrence of viviparity has also been reported recently in *S. algira*. This salamander inhabits some of the mountain ranges in Northern Morocco and Algeria. Morphological variability within the species, accompanied with a patchy distribution, has led to the description of several subspecies. Viviparity has only been observed in some populations of one subspecies, *S. a. tingitana* (Beukema *et al.*, 2010). Again, little is known, however, about the physiological and developmental characteristics of viviparity in this latter subspecies.

Viviparity in the alpine salamander *Salamandra atra* has been extensively studied and is well characterized as one of the most derived strategies within the group (see e.g., Guex and Greven, 1994; Greven, 1998). Females ovulate around 100 eggs (40-60 per oviduct), although at ovulation only one egg per oviduct is supplied with all the jelly coats required for fertilization. Therefore, only one egg is fertilized in each oviduct despite ovulating many more eggs. Development in *S. atra* is characterized by three phases. In the first lecithotrophic phase, similar to the case in *S. salamandra*, the two developing embryos first feed on egg yolks prior to hatching from their egg membranes. By hatching, these embryos have exhausted their own yolk supplies, and they have started actively feeding on the unfertilized eggs present in the uterus (oophagy), which begins the second phase of development. After this second oophagic phase, the two embryos, which are around 25-40mm in length, move to a specialized area of the uteri called the zona trophica. This area

is characterized by the presence of transformed epithelial cells, which then serve as nutrient provisions for the embryos. In this third phase of development, the embryos feed on the enlarged maternal epithelial uterine cells (epitheliophagy) with the help of specialized toothed areas in the upper and lower jaws. The extra source of nutrients supplied by the mother (matrotrophy) enables the developing embryos to complete metamorphosis within the uterus resulting in the birth of terrestrial juveniles after three or four years of gestation (depending on altitude and environmental conditions). A similar pattern of reproduction is thought to occur in *S. lanzai*, the other species of Alpine salamander (Miaud *et al.*, 2001).

The Anatolian genus *Lyciasalamandra* contains seven recognized species, although the taxonomy and systematics of this group has been highly controversial. All of the species in this genus are viviparous. However, details of the reproductive cycles for all the species are still missing for the members of this genus. The general pattern, studied in *L. luschni* (Özeti, 1979), involves an elaborated courtship in which the male inserts a protuberance at the base of its tale (“dorsal tail tubercule”) into the female cloaca (Sever *et al.*, 1997), shortly before delivering a spermatophore on the ground. Females then pick up the spermatophore and store it in the spermatheca. After ovulation, one egg per oviduct is fertilized and develops in each uterus, the remaining ovulated eggs not being fertilized. The two developing embryos will hatch from the egg membranes within the uterus, and feed on these unfertilized eggs once they have exhausted their yolk provision. After five to nine months of gestation, females will deliver two fully metamorphosed terrestrial juveniles.

Final remarks

Despite having different evolutionary origins, viviparity in salamanders presents some similar characteristics among the different species (Wake, 1992). For instance, the fertilization process has been modified in all the viviparous species, resulting in the production of many unfertilized or arrested eggs. This contrast sharply with ovoviviparous species, in which most, if not all, of the eggs are fertilized and develop normally. Embryos of viviparous species are also characterized by the early hatching within the maternal uteri. In conjunction with early hatching, they are also characterized by the precocious (heterochronic) development of the structures related to feeding in the embryos, and their early hatching within the uteri, which enables them to feed on new sources of nutrients provided by the mother (oophagy, epitheliophagy and adelphophagy).

The question remains, however, why has viviparity only evolved within this group of salamanders and not in other lineages? What are the morphological, physiological, and

developmental characteristics that have permitted the evolution of this strategy only within Salamandridae? An in-depth analysis and characterization of the reproductive strategies among the different species will shed light on the actual morphological, physiological, developmental, and genetic elements that have led to the evolution of this remarkable mode of reproduction exclusively within this salamander lineage.

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Fig. 1: Simplified phylogeny of salamander families based on molecular data from Zhang *et al.* (2009) and Pyron and Wiens (2011). Clade A, in blue, highlights the seven families presenting internal fertilization.

Fig. 2: Phylogenetic relationships among species within the family Salamandridae, based on data from Weisrock *et al.* (2006) and Zhang *et al.* (2008). The clade ‘*Newts*’ includes 71 species of highly aquatic organisms, forming a sister clade to the so-called ‘true’ salamanders. The relationships among the ‘true’ salamanders, together with their reproductive modes, are depicted in the figure: **Black:** oviparity; **Yellow:** ovoviviparity; **Red:** viviparity. The red stars designate ovoviviparous species in which viviparity has evolved in some populations or subspecies.