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A biogeographic and ecological perspective to the evolution of reproductive behaviour in the family Salamandridae

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ABSTRACT

Amphibians have a complex reproductive behaviour, which shows the highest diversity among tetrapodes. The family Salamandridae, distributed across the entire Holarctic, is one of the most diverse groups of extant salamanders comprising 114 species in 21 genera. The family has a remarkable diversity of courtship modes, amplexus and sperm transfer. It is often hypothesised that this diversity has evolved in adaptation to a specific mating and/or breeding habitat. We test this hypothesis based upon a phylogenetic reconstruction using the complete mitochondrial genome sequences of 45 Salamandridae species, representing all existing genera. We used ancestral character state reconstruction methods and geographic range models and applied relaxed Bayesian molecular clock models to discuss the results in a temporal framework of Salamandridae evolution. Our results show that the family Salamandridae started to diversify in the Late Cretaceous (ca. 87 mya) and is of Western Palearctic origin. Ancestral character state reconstruction predicts that its common ancestor was oviparous, mated on land without amplexus and probably showed a pin wheel spermatophore transfer, which is still found in the Italian endemic *Salamandrina terdigidata*. Our results suggest that several colonization of continents with subsequent radiations took place, once to the Nearctic and twice into Eastern Asian realms. However, these events were only in one case associated with a change in mating behaviour (dorsal amplexus in Nearctic newts). Around the Cretaceous-Paleogene boundary (K-Pg boundary) several Salamandridae lineages further diverged, again with no obvious changes in mating behaviour. Overall, there is no significant signal for mating character evolution being caused by changes in habitat type, with only a slight tendency that changes in mating habitat might have led to changes in the type of sperm transfer which in turn was associated with changes in the presence or absence of amplexus.

1. Introduction

Identifying the major drivers of character change is inevitably coupled with the question of which factors favour the long-term maintenance of the respective ancestral trait (Gomez-Mestre et al., 2012). Understanding trait evolution is particularly challenging when it comes to reproductive modes, which may evolve through an interplay of natural and sexual selection. Utilization of new habitats in the course of evolution may force organisms to adapt new reproductive strategies, while intraspecific social competition may act as a potent selective force which finally shapes the behaviour and morphology of organisms (West-Eberhard, 1983).

Amphibians have the highest diversity in reproductive modes among tetrapodes (e.g. Haddad and Prado, 2005; Jamieson, 2003; Sever, 2003). This tremendous variety is mainly confined to Anura, but among Urodela the family Salamandridae shows also a remarkable diversity in courtship modes, types of amplexus (mating embrace of the female by a male) and sperm transfer (Sparreboom, 2014). The Salamandridae are one of the most diverse groups of extant salamanders, comprising 114 species in 21 genera (AmphibiaWeb; last inquiry 21.09.2016). Salamandrids were formally subdivided into two groups, the true salamanders (genera *Chioglossa*, *Mertensiella*, *Lyciasalamandra* and *Salamandra*) and newts (all other genera). However, newts have recently been shown to be paraphyletic, with the Italian endemic *Salamandrina* representing the sister lineage of a joint true salamander and newt clade (Zhang et al., 2008).

The Salamandridae are distributed across the entire Holarctis (Weisrock et al., 2006) with the highest species diversity occurring in Europe and Asia (Sparreboom, 2014). Most salamandrids show a biphasic life cycle, but some are permanently aquatic (e.g., *Pachytriton*),

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[1] Our co-author Max Sparreboom passed away during the final stage of manuscript preparation; we dedicate this paper to him.

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while others strictly terrestrial (e.g., *Echinotriton, Lyciasalamandra* and *Salamandra*) (Sparreboom, 2014). These different life cycles are related to the mating habitat. True salamanders and the genus *Salamandrina* mate on land, while newts mate in water. The sole exception within the true salamanders is *Mertensiella caucasica*, which is mating on land and in water (Sparreboom, 2014), and within the newts the genus *Echinotriton* which mate on land and the species *Tylototriton shanjing* and *T. taliangensis* which partly mate on land (Sparreboom, 2014, Sparreboom et al., 2001, Ye et al., 1993).

Because of their biphasic life cycles the most common reproductive mode in the Salamandridae is oviparity (Sparreboom, 2014). In some terrestrial species viviparity, which is generally rare among urodeles (Wake, 1993), is assumed to have evolved as an adaptation to life on land (*Lyciasalamandra* and *Salamandra*; Buckley, 2012; García-París et al., 2003; Veith et al., 1998). Courtship behaviour and reproductive modes are remarkably diverse in the Salamandridae (Houck and Arnold, 2003; Salthe, 1967; Sever, 1992; Sparreboom, 2014). Males use chemical, visual or tactile signals to attract a female (Wells, 2007). Tactile signals are performed through different kinds of amplexi, and a variety of modes of sperm transfer have been described for Salamandridae.

Despite their complex nature, some reproductive modes and their associated courtship behaviour may have evolved several times homoplastically within the Salamandridae (e.g., Steinfartz et al., 2007), such as viviparity in *Lyciasalamandra* and some species of *Salamandra*. Intrauterine nutrition of larvae differs among species (Greven and Guex, 1994; Guex and Greven, 1994) and indicates convergent evolution. Parallel evolution is discussed for the mating behaviour of *Calotriton* and *Euproctus* (Steinfartz et al., 2007), two newt genera which are not sister taxa and which both use the restraint of the female (RF) amplexus, which refers to catching with the tail or with the mouth, probably as an adaptation to stream breading. *Echinotriton* and *Salamandrina*, which both mate without amplexus (Sparreboom, 2014), are also not closely related. These examples raise the question under which selective conditions complex behavioural reproductive traits evolved several times homoplastically. Correlation in either molecular or morphological characters is generally taken as evidence for natural selection acting on those characters (Harvey and Pagel, 1991). Salthe (1967) hypothesized that the mating habitat is of paramount importance for behavioural adaptation. In contrast, Houck and Arnold (2003) argued that ecological sites are poor predictors of salamander courtship. Rather ecological (mating) sites are more important for male-male competition or the ability to select a mating partner (Sullivan et al., 1995).

Behavioural components of the Salamandridae reproductive biology have repeatedly been studied in the context of character evolution. While behavioural character states themselves were used to infer phylogenetic relationships among Salamandridae and subclades therein initially (e.g., Arnold 1977; Arntzen & Sparreboom 1989; Halliday 1977; Halliday 1990), phylogenies based on molecular characters helped to identify homoplastic character states later (e.g., Veith et al., 1998). Steinfartz et al. (2007) analyzed the evolution of selected life history traits (reproduction and adult habitat use, degree of body contact, and male-mediated courtship display) for 38 salamandrid species, with special emphasis on the *Triturus* clade, using a phylogenetic tree based on three mitochondrial genes fragments. Wiens et al. (2011) analyzed in depth the evolution of the sexually selected dorsal crest and behavioural elements of the male display behaviour within the clade of European newts.

Here we focus on the evolution of more general ecological and behavioural aspects of Salamandridae reproductive biology, namely the basic reproductive mode, the habitat during mating, the type of amplexus and the type of sperm transfer. We study members of all genera of the family, covering their complete geographic range. We use complete mitochondrial genome sequences of 45 species of the family Salamandridae to produce a robust mitochondrial phylogeny of Salamandridae for which we estimated an evolutionary timeframe. We used this tree to test the hypothesis that the habitat is a major driver of the evolution of reproductive modes in Salamandridae (Salthe, 1967) and added geographical aspects by reconstructing the ancestral ranges of lineages. More specifically we ask: (*i*) What are the character states of the common ancestors within the family Salamandridae? (*ii*) Can significant evolutionary changes of components of mating behaviour be related to changes of habitat or ranges? (*iii*) Did different components of mating behaviour evolve in concert?

2. Material and methods

We here adopt the denomination of Zhang et al. (2008) which is based on well supported molecular clades. True salamanders comprise the genera *Chioglossa*, *Mertensiella*, *Salamandra* and *Lyciasalamandra* (see also Veith et al., 1998). The newts are divided into primitive newts (*Echinotriton*, *Pleurodeles* and *Tylototriton*), New World newts (*Notophthalmus* and *Taricha*), Corsica-Sardinia newts (*Euproctus*), modern European newts (*Calotriton*, *Ichtyhosaura*, *Lissotriton, Neurergus*, *Ommatotriton* and *Triturus*), and modern Asian newts (*Cynops, Laotriton, Pachytriton* and *Paramesotriton*). The genus *Salamandrina* does not fit into any of these categories and is therefore mentioned by its genus name.

2.1. Taxon sampling

We used the entire dataset of Zhang et al. (2008) for phylogenetic reconstruction and added six additional complete mitochondrial sequences of members of the family Salamandridae from GenBank. Thus, the complete dataset consisted of 45 salamandrid taxa representing all genera of the family. As outgroups we used *Ambystoma mexicanum*, *Andrias davidianus*, *Ranodon sibiricus*, *Rhyacotriton variegatus* and *Xenopus tropicalis* (for detailed information see Supplementary Information Table 1). Extensive literature search showed that there are no intrageneric polymorphisms of the reproductive behaviour of Salamandridae, apart from the genus *Tylototriton*. Therefore, these 45 taxa adequately represent the variability of analysed characters of Salamandridae.

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2.2. Sequence alignment and phylogenetic reconstruction

We automatically aligned the sequences with MAFFT (version 7, Katoh and Standley, 2013) using the iterative refinement method (Berger and Munson, 1991; Gotoh, 1993) and the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970) with default parameter settings. Ambiguous areas in the alignment were excluded using Gblocks (version 0.91b , Castresana, 2000) with the following settings: minimum number of sequences for a conserved position 26, minimum number of sequences for a flanking position 42, maximum number of contiguous nonconserved positions 8, minimum length of a block 10 and no gaps allowed (default settings).

We divided the entire alignment into nine partitions following Powell et al. (2013), who argued that the most useful categories for partitioning are codon position, RNA secondary structure pairing and the coding/noncoding distinction. In order to assign bases of RNA genes to the corresponding paired (stem regions) versus unpaired (loop