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The evolution of viviparity in holocene islands: ecological adaptation versus phylogenetic descent along the transition from aquatic to terrestrial environments

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Abstract

Species that contain populations with different reproductive modes offer excellent opportunities to study the transition between such strategies. *Salamandra salamandra* (Linnaeus, 1758) is one of two species within the *Salamandra–Lyciasalamandra* clade which displays two reproductive modes simultaneously. Along the *S. salamandra* distribution, the common reproductive mode is ovoviviparity although the species also has viviparous populations in the northern Iberian Peninsula. The occurrence of viviparity has recently been reported in two small offshore island populations on the Atlantic coast (NW Iberia), which originated after the last glacial period (8000–9000 years ago). In this paper, we analysed ovoviviparous, hybrid and viviparous populations (inland and mainland) from 17 localities across the northern Iberian Peninsula using two mitochondrial markers (Cyt *b* and *COI*, *c.* 1100 bp). Phylogenetic and phylogeographic analyses highly support that viviparity arose as an evolutionary novelty in the *S. salamandra* island populations and that viviparous populations are therefore not monophyletic. The recent insularity of Atlantic island populations leads us to conclude that the transition from ovoviviparity to viviparity can happen in a very short-time span. Additionally, to determine the likely source of this evolutionary transition, we discuss how ecological pressures could have an effect on the maintenance of the ovoviviparous reproductive mode. Hence, taking into account the results of this study, we propose the consideration of the island populations as an evolutionary unit for conservation purposes.

Key words: Ecological pressures – evolution – islands – mtDNA – ovoviviparity – *Salamandra* – viviparity

Introduction

The evolutionary transition from oviparity to viviparity opened a wide array of possibilities for ecological diversification and adaptive radiation (Shine 1989). This transition has been recorded in invertebrates (Kohler et al. 2004), fishes (Thibault and Schultz 1978; Wourms and Lombardi 1992; Goodwin et al. 2002), amphibians (Wake 1993), reptiles (Shine 1985; Blackburn 2000), and mammals (Tyndale-Biscoe and Renfree 1987). However, the evolutionary steps from an oviparous to a viviparous reproductive mode are not commonly taken. Developmental constrains are likely to be one of the main factors contributing to the lack of generalization of viviparity (Andrews and Mathies 2000).

Despite these constraints, viviparity in reptiles has evolved far more often than in all other lineages (Blackburn 1992). Other ectotherm vertebrates do not show such high rates. Amphibians exhibit higher diversity of reproductive modes than other tetrapod vertebrates (Haddad and Prado 2005), but viviparity occurs only widely among the Gymnophiona, and only occasionally in Anura and Urodela (Wake 1993). Within urodeles, viviparity is limited to a single clade of Eurasian salamanders: the sister genera *Salamandra* and *Lyciasalamandra* (Veith et al. 1998; Garcia-Paris et al. 2003) within the family of Salamandridade. Based on the site of egg deposition and the type of larval development, salamanders of the family Salamandridae present three general reproductive modes: *oviparity*, where females lay eggs in water and have aquatic larvae; *ovoviviparity* (using the term following Alcobendas et al. (1996), in which the developing embryos are retained in the oviducts until they become small larvae which are later laid in water; and *viviparity*, with oviductal retention of developing offspring, which are born as fully metamorphosed juveniles (Duellman and Trueb 1986; Pough et al. 2004).

The *Salamandra–Lyciasalamandra* clade includes both the ovoviviparous and viviparous taxa, but only two species display both strategies simultaneously: *Salamandra salamandra* (Alcobendas et al. 1996) and *Salamandra algira* Bedriaga, 1883 (Donaire Barroso and Bogaerts 2001). *Salamandra salamandra* ranges from the Iberian Peninsula to Ukraine and Greece (Montori and Herrero 2004). Along this distribution, the common reproductive mode is ovoviviparity, although the species also contain viviparous populations in the Cantabrian Mountains and the Cantabrian coast (*S. s. bernardezi*), and the SW Pyrenees (*S. s. fastuosa*) (referred as to Cantabrian populations from now on) where they give birth to terrestrial, fully metamorphosed individuals or display a mixed strategy (Dopazo and Alberch 1994; Alcobendas et al. 1996). Although viviparity was only known in the mountains of northern Spain and the Cantabrian coast, the occurrence of this reproductive mode has been reported in two small offshore island populations in the Atlantic coast of Galiza (NW Iberia) (Galán 2003). These islands are part of a complex set of small archipelagos that originated along the coast of Southwestern Galiza (Fig. 1), when the sea level rose after the last glacial period (8000–9000 years ago) (Dias et al. 2000). Both islands are included in the 'Atlantic Island National Park'.

Garcia-Paris et al. (2003), based on a study of cytochrome *b* mtDNA and a previous study of allozyme variation (Alcobendas et al. 1994, 1996), suggested that viviparity in northern Iberian populations of *S. salamandra* evolved only once.

Following this scenario, the origin of viviparity on the Galician island populations, could be the result of two independent hypotheses: (i) *Ancestral colonization*: If viviparity has arisen just once within the taxon, we would expect all viviparous populations to be more closely related to each other than to any ovoviviparous population. In this case, the island populations would be more related to viviparous populations

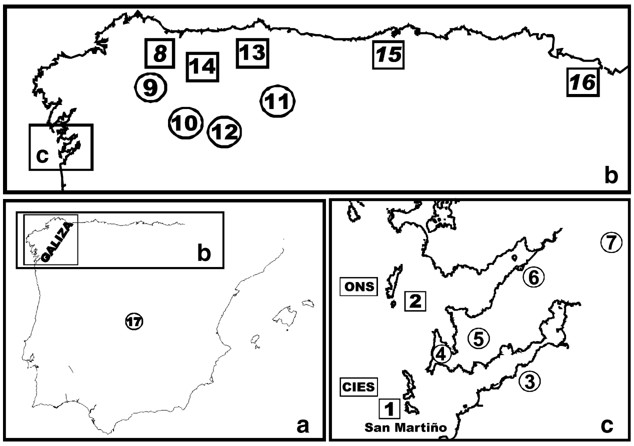

Fig. 1. (a) Map of the distribution of *Salamandra salamandra* in the Iberian Peninsula with the outgroup used in the analyses; (b) sampling locations along northern Iberia; (c) sampling localities in the Atlantic islands and closest coastal populations. Circles represent the ovoviviparous populations; viviparous populations are represented by squares, while hybrid or mixed populations are depicted in squares with numbers in italics

from Northern Iberia than to the closest mainland Galician populations. Here we test this hypothesis by a genetic comparison of the already known mainland viviparous and ovoviparous populations with the Atlantic island populations. (ii) *New origin due to ecological selection pressures*: If the islands were not colonized by viviparous populations, then island viviparity must have evolved independently, likely as a consequence of local ecological pressures (e.g. Fautin et al. 1989; Neill 1964; Packard et al. 1977; Tinkle and Gibbons 1977; Shine and Bull 1979; Shine 1989; Guillette 1993) acting over the morphological and physiological protoadaptations of *S. salamandra* (e.g. oviductal retention, D. Buckley et al. 2007).

The objectives of this paper were (1) to explore if viviparism is the only reproductive mode in the Galician island populations of *S. salamandra*, (2) to determine the genetic affinities of the island populations to the already known clades of *S. salamandra*, (3) to analyse the geographical structure of the Northwestern Iberian populations, and (4) to identify the processes driving the appearance of viviparity in the offshore islands and to tentatively date the transition from ovoviviparity to viviparity in this 'ideal' scenario.

Materials and Methods

Reproductive mode in the island populations

Among the Galician coastal islands, *S. salamandra* is only present on Ons Island and on San Martiño Island, the southernmost of the Cíes archipielago (Fig. 1). Ons and San Martiño islands are separated from the mainland by 3.6 and 6 km respectively, while 12 km of sea barrier exists between the two islands. Ons is 5 km long and 1.2 km width (428 ha), while San Martiño is 2.3 km long and 1 km width (146 ha).

Data on reproductive mode in *S. salamandra* island populations were obtained by capturing seven gravid females from Ons Island during the winters of 2004 to 2006 and maintaining them in captivity until parturition. Due to the small size of the population of San Martiño Island and to the logistic problems of accessibility, we decided not to remove any female from this island. Newly born individuals were weighed and measured as soon as possible and checked for gill presence. All females and their progeny were returned to the island. Both islands were also visited from 2004 to 2006 on several occasions from September to May searching for larvae, which would indicate the existence of alternative reproductive strategies in the islands.

Sampling for molecular analysis

We obtained tissue samples (tip of tail or finger) from 11 (San Martiño), 15 (Ons), and 9 (four coastal localities near the islands) individuals from southwestern Galiza (Fig. 1; Table 1). Additionally, 76 samples were collected in the National Museum of Natural Sciences (Madrid), from 11 localities across the northern and central plateau of the Iberian Peninsula (Fig. 1; Table 1). These localities were selected to include viviparous and ovoviviparous populations (identified earlier by genetic and morphological analyses) (Alcobendas et al. 1996; Garcia-Paris et al. 2003), a mixed population (population 16) and two hybrid populations (populations 8 and 15, Table 1; Fig. 1) with both reproductive modes in a contact zone between ovoviviparous and viviparous populations.

Table 1. Collection localities, taxonomic assignment (morphotype), sample size (n), population identifiers (ID), network haplotypes and UTM coordinates for the sequences obtained for this study

Population ID	Morphotype	Sample size	Collection locality	Haplotypes	UTM X	UTM Y
1	*S. s. gallaica*	11	Spain: Pontevedra: San Martiño Island	IV	508075	4672234
2	*S. s. gallaica*	15	Spain: Pontevedra: Ons Island	IV, V, VI	505563	4692291
3	*S. s. gallaica*	3	Spain: Pontevedra: Vigo	IX	523681	4675792
4	*S. s. gallaica*	4	Spain: Pontevedra: Melide	III	511293	4678034
5	*S. s. gallaica*	1	Spain: Pontevedra: Bueu	III	519540	4683911
6	*S. s.gallaica*	1	Spain: Pontevedra: Lourizán	IX	527753	4695721
7	*S. s. gallaica*	2	Spain: Pontevedra: San Xurxo de Sacos	IX	541951	4707104
8	*S. s. gallaica*	11	Spain: Lugo: Trabada	IX, X, XXIII, XXIV	516002	4810777
9	*S. s. gallaica*	10	Spain: Lugo: Lugo	IX, XI	545456	4762076
10	*S. s. bejarae*	2	Spain: León: Palacios del Sil	VIII	616996	4748022
11	*S. s. bejarae*	3	Spain: León: Isoba	IV	689630	4769739
12	*S. s. bejarae*	8	Spain: León: Lillo del Bierzo	IV	631799	4738230
13	*S. s. bernerdezi*	10	Spain: Oviedo: Oviedo	XIII, XIV, XV	730874	4805447
14	*S. s. bernerdezi*	10	Spain: Oviedo: Monasterio de Hermo	XVI, XVII, XVIII, XIX, XX, XXI, XXII	630909	4763424
15	*S. s. fastuosa*	10	Spain: Santander: Ucieda	I, II, VII	276684	4793457
16	*S. s. fastuosa*	2	Spain: Huesca: Bosque de Oza	XII	684139	4735508
17	*S. s. bejarae*	5	Spain: Madrid: Pelayos de la Presa	*N	381107	4467546

*N, this population has not been analysed by phylogeographic analyses.

PCR amplification and sequencing

Total genomic DNA was extracted from ethanol-preserved tissues using a phenol–chloroform protocol (Sambrook et al. 1989), preceded by a digestion with proteinase K. Polymerase chain reaction (PCR) was used to amplify 430 base pairs corresponding to part of the mtDNA Cytochrome b gene (cytb) using primers, *cytb-2* (Kocher et al. 1989) and MVZ 15 (Moritz et al. 1992), and 694 bp of the Cytochrome Oxidase I gene (COI) with primers COI H (Machordom et al. 2003) and LCO 1490 (Folmer et al. 1994). PCR reactions consisted of 40 cycles with a denaturing temperature of 94°C (1 min), annealing at 54°C (1 min) and extension at 72°C (1 min). COI annealing temperature was 49°C. All PCR reactions were performed in a total volume of 25 μl, including 0.2 μl of Taq polymerase (Biotools, 5 U ml^{-1}), 1 μl of each primer (10 mmol l^{-1}), 0.5 μl of dNTPs (10 mmol l^{-1}), 1 μl of $MgCl_2$ (25 mmol l^{-1}) and 2.5 μl of reaction buffer (Biotools, Tris-HCl, pH 8.3). Double-strand templates were cleaned using sodium acetate and ethanol to precipitate the PCR products and then re-suspended in 22 μl of ddH_2O. Sequencing reactions were performed for both the strands and sequenced on an ABI PRISM 3700 DNA sequencer following the manufacturer's instructions.

Sequence alignment and phylogenetic analyses

Sequences were read from both strands using ProSeq v.2.91 (Filatov 2002), and because the mitochondrial data displayed relatively low levels of variation, alignments were made by eye in the same program.

Phylogenetic inferences were estimated using maximum parsimony (MP), maximum likelihood (ML), Bayesian inference (BI), and minimum evolution (ME). MP and ML analyses were performed with PAUP* 4.0b10 (Swofford 2002). For MP, the heuristic search algorithm was performed with tree-bisection-reconnection (TBR) branch swapping, and 100 random addition sequences replicates. Statistical reliability of the resulting trees was assessed using nonparametric bootstrap (bs) with 1000 pseudoreplicates (Felsenstein 1985). We analysed the data to determine the best fitting substitution model for the ML analysis through the Akaike information Criterion (AIC) implemented in ModelTest 3.6 (Posada and Crandall 1998). ML analysis was done using the heuristic search algorithm. Branch support was assessed with non-parametric bootstrapping (100 pseudoreplicates). BI analysis was conducted with MrBayes 3.0 (Huelsenbeck and Ronquist 2001) run for 10^6 generations with a sampling frequency of 100 generations. From the 10,000 trees found, the first 1800 were discarded as 'burning'. Posterior clade probabilities (pp) were used to assess node support.

Trees were rooted using a population of *S. salamandra bejarae* from Pelayos de la Presa (Madrid) (pop. 17, Fig. 1). This population fell outside the 95% confidence limit for the MP connection of haplotypes (see Nested Clade Analysis below) and is genetically and geographically distant from the populations analysed in this study (Alcobendas et al. 1996).

Phylogeographic analysis

To estimate gene flow and population history among populations of *S. salamandra*, we conducted a phylogeographic analysis on Cytb-COI haplotype variation within the species. By integrating genealogical and geographical information, the Nested Clade Analysis (NCA) approach was able to differentiate between contemporary and historical processes, such as restricted gene flow, range expansion or past fragmentation (Cruzan and Templeton 2000). Relatedness among haplotypes was presented by an unrooted nested cladogram, generated using the software tcs 1.18 (Clement et al. 2000), which follows the statistical parsimony algorithm described in Templeton et al. (1992). GEODIS 2.0 (Posada et al. 2000) was used to perform the following analyses. First, a permutational contingency analysis, based on 10 000 resamples, enabled us to test for the significant association of haplotypes or clades with locality. Together with information on the geographical distribution and frequency of haplotypes, two parameters were then estimated for each clade (and/or haplotypes): Dc (clade distance) and Dn (nested clade distance). Data were interpreted with the phylogeographical inference key (last updated 11 November 2005) provided by these authors with Geodis 2.2.

Results

Reproductive mode in the islands

No larvae were found in the scarce ponds or streams present in the island during samplings from 2004 to 2006. Previous researchers were also unable to find larvae and observed viviparous reproduction in one female that gave birth to five fully developed offspring on land (Galán 2003). The seven females studied from Ons exhibited viviparity, giving birth to fully developed offspring. The number of newborns varied between 2 and 18. We found great variability in juvenile size, from 0.16 to 1.06 g. Two females gave birth to very small individuals (0.19 g in average), some of which had gill remains. We have no direct data available for San Martiño, but none of the sampling trips revealed the presence of larvae, and there is an old record of a female that also gave birth to fully developed juveniles (Galán 2003) indicating that viviparity is also present in that island.

Phylogenetic analysis

The combined nucleotide sequences of the *coxI* and cyt *b* fragments produced an alignment of 1124 bp. No insertions or deletions were found among the sequences. Of the 75 variable sites, 46 were informative under MP, defining 24 haplotypes (Table 1). The ML mean transition/transversion ratio was 3.95, with means nucleotide frequencies of 30.5 for A, 16.2 for C, 25.3 for G and 28.1 for T. The GRT + I + G model of evolution was selected by AIC in ModelTest 3.6. All tree-building methods resulted in an identical topology (ML tree depicted in Fig. 2). An MP heuristic search of the combined data resulted in 102 equally parsimonious trees of which a strict consensus tree was constructed (not shown). ML analyses recovered the lowest–log-likelihood (-lnL) score = 2214.4689.

The studied populations are grouped in two main clades, A and B. The first clade (clade A) groups together north-western Iberian ovoviviparous populations, island viviparous populations and some haplotypes from hybrid populations (MP bs 90%, BI pp 99%). Inside this heterogeneous group, a first subclade (clade A1), moderately supported (MP bs 60%, BI pp 80%) groups two haplotypes from the hybrid population of Ucieda, haplotypes from the closest coastal populations to the islands (Melide and Bueu) and a clade including the island populations plus two distant north-western ovoviviparous populations (Isoba and Bierzo). The second subclade (clade A2) (MP bs 80%, BI pp 100%) shows a haplotype from Ucieda, sister to the remaining ovoviviparous populations, including Galician populations (some of them close to the coast) and the hybrid population of Trabada. Clade B (MP bs 90%, BI 99 pp) groups viviparous populations and some samples from a hybrid population (Trabada). The first dichotomy within this clade splits the mixed population in the Pyrenees (Oza) from the viviparous populations from Cantabrian Mountains and the other two Trabada haplotypes.

Phylogeography

Haplotypes from Pelayos de la Presa fall outside the 95% confidence limit for the MP connection of haplotypes in the phylogeographic analysis. The remainder haplotypes were collapsed in a network with 24 haplotypes within a 5-step clade

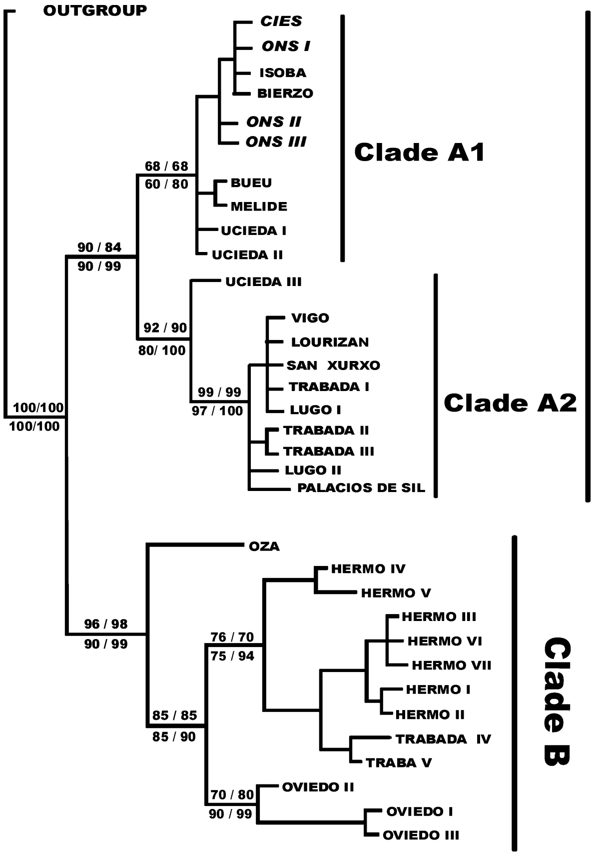

Fig. 2. *Salamandra salamandra* ML tree of mtDNA haplotypes. Numbers above nodes are bootstrap values > 60 obtained under ME and ML, number below nodes MP bootstrap values > 60 and posterior probabilities from Bayesian analysis. We only show bootstrap values discussed in the text. Vertical lines represent the main clades and subclades discussed in the text. Populations in italics within A1 subclade, represent the island population's haplotypes

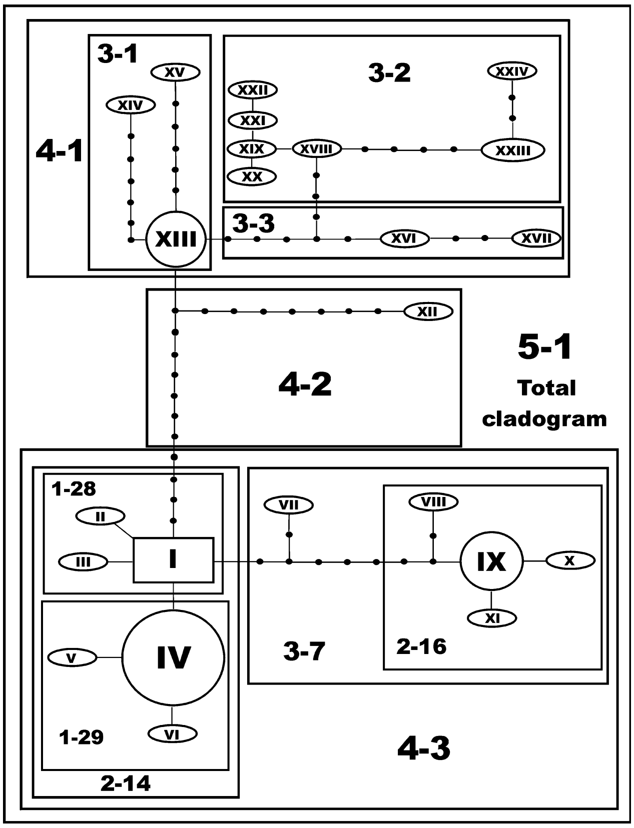

Fig. 3. Nested design for the statistical parsimony haplotype network of *COI* fragment and *cytb* fragment sequences from *Salamandra salamandra* in this study

(Fig. 3). Ten of the 63 clades generated in the network showed geographic and genetic variability which were tested for significant association between geographic and genetic variability with Geodis. In eight of 10 nested clades, the null hypothesis was rejected, demonstrating association between geographic location and genetic variability. In these eight nested clades, Dc and Dn values were interpreted following Templeton's inference key (Table 2). For clade 1–28, including haplotypes I and II (Ucieda) and haplotype III (from the Atlantic coast populations, Bueu and Melide) the analysis inferred insufficient genetic evidence to discriminate between long distance movements and the combined effects of gradual movement during a past range expansion and fragmentation. The same occurs for clade 3–7 which represents the most northwestern populations (haplotypes IX, X and XI) and haplotypes VIII (Palacios de Sil) and VII (Ucieda). For the clade 2–14 including clade 1–28 and haplotypes IV (Isoba, Bierzo and island viviparous populations), V and VI (also representing Ons Island) restricted gene flow with isolation by distance was inferred. The inference key suggests a contiguous

Table 2. Results of the Nested Clade Analysis (NCA) showing all clades with significant associations between geographic and genetic structure, the inference chain for each clade showing and the processes inferred by the latest version of the GeoDis inference key

Clade	Inference chain	Inferred processes
1–28	1-2-11-12-13-14-21	Insufficient genetic evidence to discriminate between long distance movements and the combined effects of gradual movement during a past range expansion and fragmentation (Ucieda, Melide-Bueu)
2–14	1-2-3-4	Restricted gene flow with isolation by distance (Ucieda, Melide-Bueu, Ons-Cies-Isoba-Bierzo)
2–16	1–2	Inconclusive outcome
3–2	1-2-11-12	Contiguous range expansion (Hermo, Trabada)
3–7	1-2-3-5-6	Insufficient genetic evidence to discriminate between long distance movements and the combined effects of gradual movement during a past range expansion and fragmentation (Ucieda, Palacios de Sil, Trabada, Lugo, Pontevedra)
4–1	1-2-11-12	Contiguous range expansion (Oviedo, Hermo, Trabada)
4–3	1-2-3-4	Restricted gene flow with isolation by distance
Total cladogram	1-2-3-5-6	Insufficient genetic evidence to discriminate between long distance movements and the combined effects of gradual movement during a past range expansion and fragmentation

range expansion for clades 3–2 and 4–1 (Table 2). An inconclusive outcome was inferred for clade 2–16 (haplotypes VIII, IX, X and XI). Finally, for the total cladogram the analysis inferred insufficient genetic evidence to discriminate between long distance movements, and the combined effects of gradual movement during a past range expansion and fragmentation.

Viviparous mainland populations (haplotypes within clades 4–1, Fig. 3) display much higher diversity than ovoviviparous, hybrid and viviparous island populations. We found 13 viviparous haplotypes from 22 samples analysed in Cantabrian populations. In contrast, despite the large geographic distance, only 11 haplotypes characterized the 86 samples from ovoviviparous, hybrid and viviparous islands populations. Also the Trabada hybrid population (population 8, Fig. 1) had high diversity, with four haplotypes in 11 samples. Our results are in agreement with previous studies (Dopazo et al. 1998) where populations located within 100 km range (Oviedo and Hermo, clade 4–1) show the most divergence in mtDNA haplotypes. Phylogeographic analyses inferred a contiguous range expansion among viviparous Cantabrian populations (clade 4–1) and the Galician hybrid population (Table 2, Fig. 2).

Discussion

Origin of viviparity in the islands

Our results confirm that *S. salamandra* is viviparous on the Galician Atlantic islands. This is shown by laboratory parturitions from Ons females, and by the absence of larvae on the island surface water. The phylogenetic hypotheses presented here show that island and mainland viviparous clades do not form a monophyletic group. In fact, the island viviparous populations cluster in a strongly supported group (clade A) with ovoviviparous populations and two hybrid populations (Trabada and Ucieda), while mainland viviparous populations and the mixed Oza population group in clade B. Island viviparous populations do not differ morphologically from the mainland ovoviviparous Galician populations, but they differ consistently (coloration pattern, body shape and size) from the northern viviparous salamanders (Bass and Gasser 1994; Alcobendas et al. 1996). Garcia-Paris et al. (2003) mentioned a correlation between external morphology and reproductive strategies: adults of viviparous populations are small or medium sized, with a striped pattern, whereas adults of ovoviviparous populations are large and are typically spotted rather than striped. However, this is not the case for the islands populations. The external morphology data are thus congruent with the molecular results presented in this study.

Galician haplotypes cluster in two clades (clades A1 and A2; Figs 1 and 2; Table 1). Island viviparous populations (Ons, Cíes) and the nearest coastal ones (Melide, Bueu), do not cluster with the mainland Galician populations (Lugo, Vigo, …), but instead with some Cantabrian haplotypes from ovoviviparous (Bierzo), or hybrid (Ucieda, Isoba) populations. Haplotypes from Island and from their nearest coastal populations (clade A1) are surrounded by populations from a different lineage (clade A2) and are therefore isolated from their close Cantabrian relatives (clade A1). In such a scenario, considering that *S. salamandra* cannot cross the 3 km of seaway between the islands and the coast, the present populations of *S. salamandra* in these Atlantic Islands represent the fragmented remnants of ancient populations that were continuous with those of the closest coast in the Holocene. Then, the sea level rose after the last glacial period (8000–9000 years ago) (Dias et al. 2000) keeping isolated the populations of the islands. The replacement of the mitochondrial lineage A1 by representatives of lineage A2 in the lands between the coast and the Cantabrian Mountains may be a recent phenomenon. This is further supported by the associations inferred by Nested Clade Analyses with haplotypes from Ucieda population considered as the source for the Galician haplotypes (Table 2 and Fig. 3).

If colonization of the islands occurred before their isolation by the rising of the Atlantlic Ocean during the Holocene, the hypothesis that considers viviparity as a novelty in the island populations of *S. salamandra*, becomes the most realistic. In addition, Garcia-Paris et al. (2003) suggested that the migration of Cantabrian viviparous populations occurred towards the east, but not to the west, so these populations were not able to reach the Galician southwestern coast. However, phylogeographic results from this study (Table 2) confirm that viviparous mainland haplotypes could have arrived the eastern Galician population (the Trabada hybrid population).

Time frame for the ovoviviparity–viviparity transition

The recent insularity of Atlantic island populations of *S. salamandra* and the confirmation of viviparity as a novelty in these islands, let us conclude that transition from ovoviviparity to viviparity can be very fast (probably rather < 8000 years).

Moreover, island viviparous populations were isolated at the same time and viviparity appeared after the island arose. This information suggests that viviparity in island populations of *S. salamandra* has independently evolved two times. Viviparity in *S. salamandra* is characterized by the early hatching of the embryos within the maternal oviducts coupled with oophagy and adelphophagy (i.e. the intra-oviductal ingestion of eggs or larvae respectively (Wourms 1981; see Joly 1968; Dopazo and Alberch 1994; Greven 1998). D. Buckley et al. (unpublished data) studied heterochronic modifications of ovoviviparous and viviparous salamanders from one of our viviparous populations (Oviedo). The viviparous reproduction had a general acceleration of the development (taking 90 days for viviparous and 120 days for ovoviviparous until metamorphosis). This acceleration was in part due to intrauterine cannibalism but also occurred during the pre-hatching stage. Such oviductal retention is an ancestral characteristic in *Salamandra–Lyciasalamandra* clade and is present in all species within the genus.

Transition from ovoviviparity to viviparity seems to evolve readily in island populations. Therefore, physiological changes towards viviparity, in these islands, will be produced by an extreme oviductal retention requirement. However, due to the recent nature of this physiological change, it could be possible to find various degrees of viviparity in the same population, with females laying fully metamorphosed juveniles and juveniles with conspicuous gills, as occurs in mountain populations of *S. s. bernardezi*, *S. s. fastuosa* and some females in this study. Physiological analysis in viviparous populations of the islands would be necessary to compare among viviparous populations on *S. s. bernardezi* and *S. s. fastuosa*. However, we can conclude that transition to viviparity in *S. salamandra* is faster and more frequent than expected.

Ecological factors

Salamandra salamandra is a euryoic taxon: (i) it has a widespread distribution covering western and central Europe, (ii) it inhabits very different habitats (both in Mediterranean and Euro Siberian climates), and (iii) it has an extraordinary morphological diversification with at least nine subspecies recognized in the Iberian Peninsula (Montori and Herrero 2004). However, viviparity only occurs in populations within the Cantabrian Mountains, the Pyrenees and Atlantic islands (Ons and Cíes) in NW Spain.

What ecological factors might have contributed to the transition to viviparity? The evolution of viviparity has been attributed to selective forces such as environmental factors or biotic traits (e.g. Neill 1964; Packard et al. 1977; Tinkle and Gibbons 1977; Shine and Bull 1979; Shine 1985; Guillette 1993; Qualls and Shine 1998). Since this evolutionary step completely removes the aquatic stage, the obvious responsible agent of this microevolutionary event could be the absence of water in which to lay the larvae, a plausible scenario in some of the islands (Galán 2003). Interestingly, 'Cíes' originates for some authors from the Latin 'siccus', which means 'dry' (González-Alemparte 2003), and today water is scarce in these islands. The lack of available surface water in karstic limestone substrates has also been suggested as the main cause that promotes viviparity in the Cantabric Mountain *S. s. bernardezi* populations (Garcia-Paris et al. 2003). However, the presence of another amphibian [*Lissotriton boscai* (Lataste, 1979)] in all water bodies in Ons Island suggests that water limitation was not so drastic at least in Ons. On the other hand, the newts *Lissotriton helveticus* (Razoumowsky, 1789) and *Triturus marmoratus* (Latreille, 1800) which are present on the closest coast do not occur in the islands, perhaps due to the absence of favourable habitats, and *L. boscai* is found only in two islands of the National Park (Ons and Salvora, but not Cíes).

Qualls et al. (1995) reported that distributions of viviparous and oviparous reproduction modes in a lizard, *Lerista bougainvillii* (Gray, 1839), follow the 'cold climate hypothesis' on the evolution of reptilian viviparity (for review see Shine 1985; Heulin et al. 1991, 1997). The oviparous lineage in this species occurs in the south-western mainland Australia, while viviparous lineages appear in colder climates: two islands separated by approximately 1000 km. Another study testing the cold-climate hypothesis in horned lizards (*Phrynosoma*) partially supports the hypothesis (Hodges 2004). Special climate characteristics found in Atlantic islands place them inside the Mediterranean climate, which is warmer and dryer than the Atlantic climate, found in the nearby coast and northern Iberia. Viviparous clades of *S. salamandra* occur in both climates in contrast with the above hypothesis. Nevertheless, selective forces playing a role in the evolution of viviparity are well documented in reptiles. Microevolutionary change in the reproductive mode in *S. salamandra* and extinction events can happen faster in the Atlantic islands than in other places due to specific ecologic pressures acting on salamanders on these small islands (Foufopoulos and Ives 1999). One of the viviparous populations which is really threatened is on San Martiño Island, where only dozens of individuals survive in a small area. If the current conditions do not change dramatically the near future of this population is likely extinction, which will contribute to the loss of this recently emerged viviparous lineage.

To conclude, our results indicate that the salamander population inside the Atlantic Islands National Park have independently evolved the ability to reproduce viviparously. The absence of *S. salamandra* in the other islands of the Cíes archipelago seems a consequence of a recent population decline (Galán 2003). The island populations, although included within the same taxonomic unit as mainland Galician populations (*S. s. gallaica*), should be considered evolutionarily significant units (Moritz 1994) reservoir of a singular evolutionary process, and therefore of great conservation value.

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Resumen

Evolución del viviparismo en islas del Holoceno: Adapatación ecológica versus descendencia filogenética durante la transición de medios acuáticos a terrestres.

Las especies que contienen poblaciones con diferentes modos de reproducción, ofrecen las mejores oportunidades para estudiar la transición entre ambas estrategias. *Salamandra salamandra* es una de las dos especies dentro del clado *Salamandra-Lyciasalamandra*, la cual muestra simultáneamente los dos modos de reproducción. A lo largo de la distribución de *S. salamandra*, el ovoviviparismo es el modo común de reproducción, aunque también presenta poblaciones vivíparas en el norte de la Península Ibérica. Sin embargo, la aparición del viviparismo ha sido citada recientemente en poblaciones de dos pequeñas islas continentales situadas cercanas a la costa atlántica (Noroeste de la Península Ibérica), originadas después del último periodo glacial (hace 8000–9000 años). En este artículo, analizamos mediante marcadores mitocondriales (*Cyt b* y *COI*, ~1100 pb) poblaciones ovovivíparas, híbridas y vivíparas (costeras y del interior) procedentes de 17 localidades a lo largo del norte de la Península Ibérica. Los análisis filogenéticos y filogeográficos corroboran un origen del viviparismo como novedad evolutiva en las poblaciones insulares de *S. salamandra*, descartando por lo tanto, la monofilia de las poblaciones vivíparas. A su vez, la reciente insularidad de las islas Atlánticas, nos permiten estimar que la transición del ovoviviparismo al viviparismo puede ocurrir en un breve periodo de tiempo. Igualmente, para determinar la posible causa de esta transición evolutiva, discutimos la posibilidad de cómo determinadas presiones ecológicas pudieron haber afectado al mantenimiento del ovoviviparismo como estrategia reproductora. Por lo tanto, y teniendo en cuenta los resultados de este estudio, proponemos considerar a las poblaciones isleñas como una unidad evolutiva para propuestas de conservación.

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