DOI: 10.1111/jbi.13192

RESEARCH PAPER

WILEY Journal of Biogeography

Role of colonization history and species-specific traits on contemporary genetic variation of two salamander species in a Holocene island-mainland system

André Lourenço[1,2] | Fernando Sequeira[1] | David Buckley[3] | Guillermo Velo-Antón[1]

[1]CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto, Instituto de Ciências Agrárias de Vairão, Vairão, Portugal

[2]Departamento de Biologia da Faculdade de Ciências da Universidade do Porto, Porto, Portugal

[3]Departamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales MNCN-CSIC, Madrid, Spain

Correspondence
Guillermo Velo-Antón, CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto, Instituto de Ciências Agrárias de Vairão, Vairão, Portugal.
Email: guillermo.velo@cibio.up.pt

Funding information
FEDER; Foundation for Science and Technology, Grant/Award Number: PTDC/BIA-EVF/3036/2012, POCI-01-0145-FEDER-006821, FCOMP- 01-0124-FEDER-028325, IF/01425/2014, PD/BD/106060/2015, UID/BIA/50027/2013

Abstract

Aim: Investigate the role of colonization history and life history traits on contemporary patterns of genetic variation in two salamander species in a Holocene island-mainland system.

Location: Rías Baixas, north-western Spain.

Methods: We analysed mitochondrial and species-specific nuclear markers (eight microsatellite markers) in 16 populations of *Salamandra salamandra* and *Lissotriton boscai*. Contemporary gene flow patterns between mainland and islands populations were evaluated by migration analyses, whereas approximate Bayesian computation (ABC) was used to assess colonization history of insular populations of both species.

Results: Land bridge populations of both species exhibited reduced genetic diversity and increased genetic structure compared to mainland populations. ABC analysis showed that insular populations of both species were established by vicariance rather than by colonization via dispersal. We did not find evidence for contemporary gene flow, though the *L. boscai* insular population of Sálvora showed genetic admixture with mainland populations.

Main conclusions: This study supports the role of genetic drift in driving contemporary genetic variation of small and isolated populations. Other interplaying factors (e.g. island size, bathymetry) seemed to influence genetic variation, highlighting the importance of integrative studies to better understand the evolutionary dynamics of land bridge populations of amphibians.

KEYWORDS
Approximate Bayesian Computation, colonization history, Galician Atlantic islands, genetic drift, land bridge islands, life history traits, *Lissotriton boscai*, *Salamandra salamandra*

1 | INTRODUCTION

During the transition from Late Pleistocene to Middle Holocene, the average global sea level increased considerably (ca. 120 m; Lambeck, Rouby, Purcell, Sun, & Sambridge, 2014). As a result, vast coastal lowlands were flooded, while mountain/hill tops on the edge of the continental shelf became separated from the mainland by seawater, thus generating land bridge islands. During this process, many populations became isolated on these islands and diverged vicariantly from their mainland counterparts. The cessation of gene flow with continental populations, together with the reduction of the effective population size (*Ne*), exacerbated the effects of genetic drift, resulting in reduced genome-wide diversity and increased genetic differentiation compared with mainland populations (e.g. Frankham, 1997; Hurston et al., 2009; Velo-Antón, Zamudio, & Cordero-Rivera, 2012; Yoichi, Jin, Peng, Tamaki, & Tomaru, 2017).

However, in non-flying species, some individuals may also disperse overseas and establish new insular populations (hereafter colonization via dispersal; e.g. Bell, Drewes, & Zamudio, 2015; Funk et al., 2016). Both vicariance and colonization via dispersal imply a reduction of *Ne*, but depletion of genetic diversity and increase of genetic differentiation is expected to be higher in the latter scenario due to a lower initial *Ne* (Frankham, 1997).

Amphibians are regarded as saltwater intolerant due to their highly permeable skin (Duellman & Trueb, 1994), rendering seawater a putative barrier to dispersal. Previous studies of land bridge amphibian populations reported pronounced genetic differentiation and reduced allelic diversity compared to their mainland counterparts, highlighting the effects of vicariance (e.g. Bell, Brasileiro, Haddad, & Zamudio, 2012; Bessa-Silva et al., 2016; Martínez-Solano & Lawson, 2009; Velo-Antón et al., 2012; Wang et al., 2014). Nevertheless, colonization via dispersal (possibly via rafting) of oceanic islands (Bell et al., 2015; Measey et al., 2007; Vences et al., 2003) has been shown in amphibians. Excluding potential cases of anthropogenic translocation (e.g. Martínez-Solano & Lawson, 2009), these studies indicate that some amphibians may be at least moderately tolerant to seawater. Physiological mechanisms to cope with osmotic stress in amphibians, regardless of their underlying causes, have been observed mostly in coastal populations, which have a greater likelihood of contacting brackish waters (e.g. Gomez-Mestre & Tejedo, 2003; Hopkins & Brodie, 2015; Hopkins et al., 2016). Hence, species highly dependent on aquatic systems to feed, disperse and mate are more likely to evolve such mechanisms and disperse overseas from coastal regions than those displaying a predominantly terrestrial life style (Hopkins & Brodie, 2015).

Building coalescent model frameworks contrasting explicitly plausible colonization scenarios (vicariance versus colonization via dispersal), and acknowledging putative species-specific life history traits that might have influenced species' demographic history may provide a better understanding of the evolutionary processes shaping contemporary genetic variation in land bridge amphibian populations. Bearing this in mind, we studied two urodele species—the fire salamander (*Salamandra salamandra*, Linnaeus 1758) and the Bosca's newt (*Lissotriton boscai*, Lataste 1879)—in a Holocene island-mainland system located in the north-western (NW) Iberian Peninsula. *Salamandra salamandra* populations likely inhabited the NW Iberian Peninsula before the Pleistocene (García-París, Alcobendas, Buckley, & Wake, 2003; Velo-Antón, García-París, Galán, & Cordero Rivera, 2007), whereas *L. boscai* arrived from the Iberian Central Mountains during the Late Pleistocene (ca. <20 kya; Teixeira et al., 2015). These species are also dissimilar in life cycles and habitat use. *Salamandra salamandra* is characterized by a larviparous reproductive mode (along most of its distribution range) in which an aquatic larval stage (up to ca. 90 larvae; Velo-Antón, Santos, Sanmartín-Villar, Cordero-Rivera, & Buckley, 2015) is followed by metamorphosis into a terrestrial adult. This confers an almost complete independence from water bodies, because females move to ponds or rivers only to deliver the larvae. Furthermore, *S. salamandra* evolved to viviparity (live-bearing) in these islands, delivering ca. 1–35 fully metamorphosed terrestrial juveniles (Velo-Antón et al., 2007, 2015), further increasing their independence from water. Conversely, *L. boscai* females are oviparous, laying ca. 100–250 eggs in subaquatic vegetation (Díaz-Paniagua, 2002). Adults spend most of their life cycle in a diverse range of freshwater systems (e.g. ponds, streams, rivers, lagoons, human-made structures), despite also occupying heterogeneous terrestrial habitats. This higher dependence on aquatic systems may have implications for marine dispersal in coastal areas, because species exhibiting a predominant aquatic life style are more likely to develop salt-tolerant phenotypes (Hopkins & Brodie, 2015). Although tolerance to salinity was not investigated here, previous studies reported salt-tolerant coastal populations of closely related *Lissotriton* species inhabiting similar environments to our study area (*L. helveticus* and *L. vulgaris*; Hopkins & Brodie, 2015); such phenotypes have not been reported for *Salamandra* species. This putative higher ability for overseas dispersal, together with the recent colonization of the NW Iberian Peninsula, lead us to hypothesize that colonization via dispersal is more likely to have occurred in *L. boscai* than *S. salamandra*.

We aim to (1) characterize contemporary patterns of genetic diversity and structure in insular and mainland populations of both species, (2) use approximate Bayesian computation (ABC) to determine which colonization processes (vicariance versus colonization via dispersal) originated insular populations of both species and (3) discuss the putative influence of species-specific life history traits on the demographic history and contemporary genetic variation of land bridge populations. We hypothesize that (H1) insular populations of both species are genetically less diverse and more differentiated than mainland populations; and (H2) our ABC framework will provide higher statistical support for a vicariance model in *S. salamandra* and for a colonization via dispersal scenario in *L. boscai*.

2 | MATERIALS AND METHODS

2.1 | Study area and sampling

Our study was carried out in an Atlantic land bridge island system, within the Galician Atlantic Islands National Park, located in the region of Rías Baixas in NW Spain (Figure 1). These small off-shore continental islands originated when coastal mountain tops became isolated due to the rising sea level during the Holocene, approximately 6–13 kya (Dias, Boski, Rodrigues, & Magalhães, 2000; Figure 2a). This system is composed of three small archipelagos: (1) the Cíes archipelago, formed by San Martiño (146 ha), Faro (106 ha) and Monteagudo islands (182 ha); (2) the Ons archipelago, which includes the islands of Ons (428 ha) and Onza (32 ha); and (3) the Sálvora archipelago, encompassing the island of Sálvora (186 ha) surrounded by several small islets. The archipelagos are 10–20 km from each other and <6 km from the mainland. Bathymetric data and the presence of small islets between the continent and Sálvora suggest that this island originated later (Figures 1 and 2a, panels a1 and a2). The sampled localities of Grove and Monteferro are located in small peninsulas. When the sea reached its maximum level (i.e. contemporary sea level) 3.5–6 kya (Dias et al., 2000), these localities likely separated from the mainland, comprising land bridge islands (panel a3 of Figure 2a; see Velo-Antón

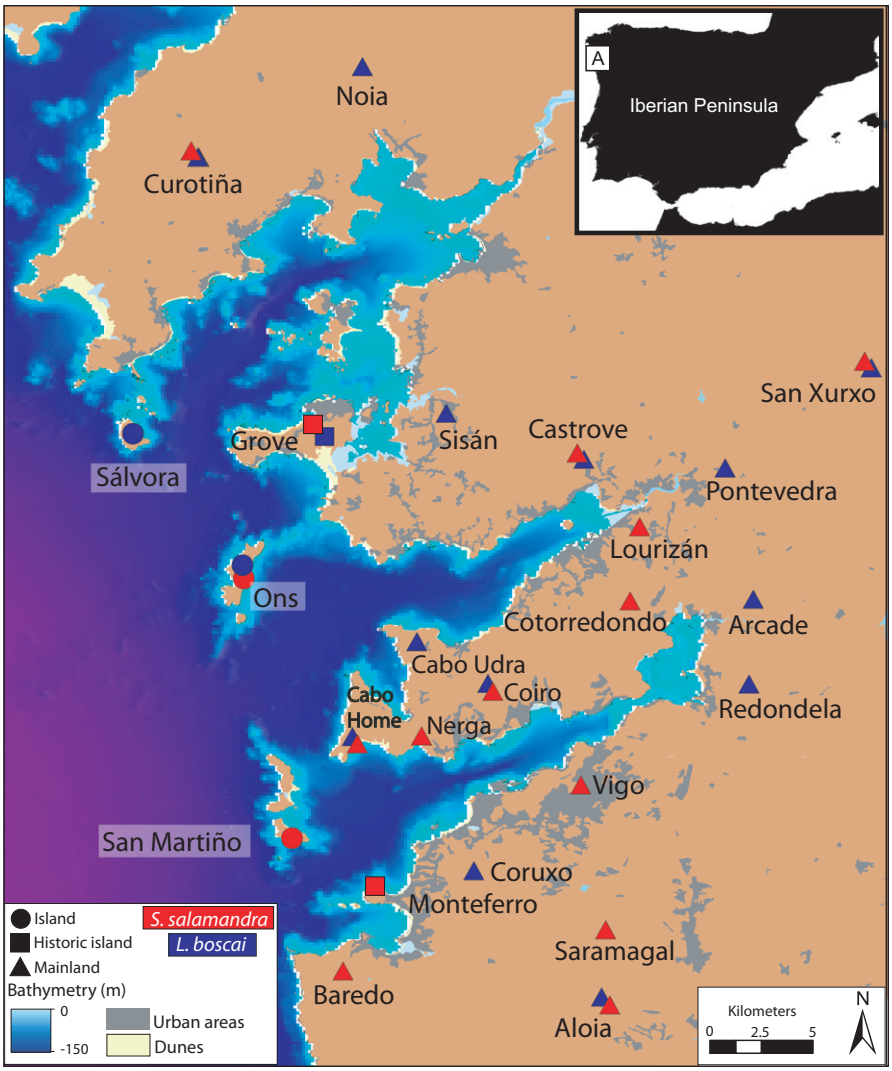

FIGURE 1 Sampled localities of *Salamandra salamandra* and *Lissotriton boscai* in the study area (north-western Spain). Red and blue symbols correspond to sampled localities of *S. salamandra* and *L. boscai*, respectively. Circles represent localities that are currently islands; squares highlight sampled localities that historically might have been islands. Triangles correspond to sampled localities in the mainland. Bathymetry is also represented, where lighter blue areas correspond to lower bathymetric zones

et al., 2012). However, a continuous sedimentation process about 2 kya along the Atlantic coast (Dias et al., 2000) re-connected them to the continent (panel A4 of Figure 2a)—consequently we refer to these as "historical islands." Mainland sampling localities of both studied species are mostly located in patchy forested areas that are likely well connected to other suitable habitats.

We sampled the extant insular and closest neighbouring localities in the mainland of both urodeles (Figure 1). The two species do not coexist in all three archipelagos: *S. salamandra* is present only on San Martiño and Ons, whereas *L. boscai* inhabits Ons and Sálvora. Sampling comprised the two extant insular populations of both species, two coastal mainland localities that may historically have been islands (Grove and Monteferro; *L. boscai* is absent in the latter), and 12 and 13 mainland localities for *S. salamandra* and *L. boscai*, respectively (Table 1). In total, 222 tail or toe clip samples of *L. boscai* were collected for genetic analyses. For *S. salamandra*, a total of 369 samples were collected, of which 224 were genotyped in a previous study (Velo-Antón et al., 2012). Individuals were released back to their sampling location following tissue sampling. Tissues were preserved in 95% ethanol at room temperature until further analyses.

2.2 | Laboratory procedures

We extracted genomic DNA using the EasySpin Genomic DNA Minipreps Tissue Kit, following the manufacturer's protocol. Quality of extract products was assessed in a 0.8% agarose gel.

We amplified and sequenced fragments of the mitochondrial DNA (mtDNA) genes NADH dehydrogenase subunit 4 (*NAD4*) and cytochrome b (cyt *b*) for *L. boscai* and *S. salamandra* respectively. These mtDNA markers were successfully used to investigate the evolutionary history of *L. boscai* (Martínez-Solano, Teixeira, Buckley, & García-París, 2006; Teixeira et al., 2015) and *S. salamandra*

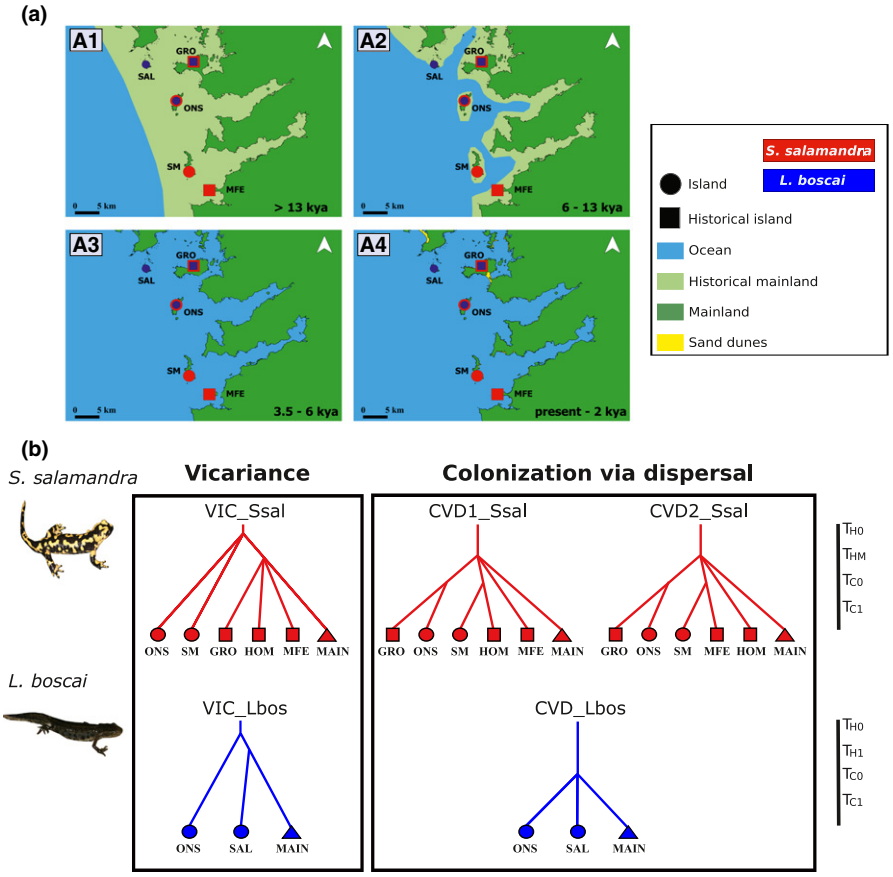

FIGURE 2 Putative evolutionary dynamics of the studied populations. *Salamandra salamandra* populations are represented by red symbols and *Lissotriton boscai* populations by blue symbols. Circles, squares and triangles correspond to insular populations, coastal populations and the mainland deme respectively. (a) Schematics showing putative patterns of sea level variation in the study area (Rías Baixas, north-western Spain) based on coastal morphology (reviewed in Dias et al., 2000). (a1) At more than 13 kya, all populations were connected to the continental shelf. (a2) Between 6–13 kya, the sea level rose considerably, flooding lowlands. The land bridge islands of Ons (ONS) and San Martiño (SM) became separated from the mainland by seawater. Sálvora (SAL) likely remained connected to the mainland during this period based on bathymetric data. (a3) Maximum sea level (i.e. contemporary sea level) was reached around 3.5–6 kya. Some coastal populations, such as Grove (GRO) and Monteferro (MFE) likely became separated from the mainland, thus generating temporary land bridge islands. (a4) Around 2 kya, a continuous sedimentation process along the Atlantic coast re-connected GRO and MFE to the mainland (Dias et al., 2000). (b) Demographic scenarios tested in the ABC framework, which illustrate putative vicariant (VIC) and colonization via dispersal (CVD) processes in both species. Most T parameters reflect population divergence (T_{HO}, T_{H1}, T_{HM}, T_{CO}), while T_{C1} represents an increase in the *Ne* following the colonization of islands at T_{C0} (illustrated by the increase in tree branch thickness). Note that scenarios CVD1_Ssal and CVD2_Ssal are nearly identical, only differing in the colonization of SM from HOM or from MFE, respectively. Time is not to scale

(García-París et al., 2003; Velo-Antón et al., 2007). Detailed laboratory procedures employed to amplify and sequence mtDNA markers are available in Appendix S1. For *L. boscai*, we used the primers ND4 and Leu (Arévalo, Davis, & Sites, 1994) to amplify ca. 700 bp of *NAD4*. For *S. salamandra*, we used the primers Glu14100L and Pro15500H (Zhang, Papenfuss, Wake, Qu, & Wake, 2008) to amplify ca. 1400 bp of cyt *b*. We verified, aligned and manually corrected the obtained chromatograms using Geneious 4.8.5 (http://www.geneious.com/). The alignment of cyt *b* was trimmed to avoid missing data, resulting in a consensus sequence of 940 bp.

Eight previously characterized microsatellites were amplified and genotyped for each species: E2, E6, E7, E8, E11, E14, S3 and S23 for *S. salamandra* (Steinfartz, Kuesters, & Tautz, 2004) and Ltb4, Ltb11, Ltb12, Ltb17 and Ltb28 (Sequeira, Silva-Ferreira, & Lopes, 2012) and Ltb25, Ltb31 and Ltb37 (Peñalver-Alcázar, Martínez-Solano, Sequeira, & Aragón, 2017) for *L. boscai*. See Appendix S1 for details about laboratory procedures.

2.3 | Phylogeographical analyses

We used the mtDNA fragments to assess phylogeographical patterns in NW Iberia. For *S. salamandra*, we used 36 samples from the 16 sampled localities located in the studied island-mainland system and 34 from other localities in the NW Iberian Peninsula where the lineage *S. s. gallaica/bejarae* is distributed. A total of 33 new *L. boscai* sequences, obtained from 14 localities in the focal area, were pooled with 45 published mtDNA sequences from NW Iberian populations (Teixeira et al., 2015). We constructed haplotype networks using statistical parsimony, as implemented in tcs 1.21 (Clement, Posada, & Crandall, 2000).

TABLE 1 Information concerning sampled populations of *Salamandra salamandra* and *Lissotriton boscai* in the island-mainland system of Rías Baixas, north-western Spain (Pop ID, population's acronym; Lat, latitude; Long, longitude; *N*, sample size). Populations are sorted out by sampling location (IS, island; H-IS, historical island; MAIN, mainland). Asterisks denote localities in which just one species was sampled. Standard genetic statistics are also displayed: H_O, observed heterozygosity; H_E, expected heterozygosity; A_R, allelic richness; *R*, mean relatedness. *N* denotes the samples size and mtDNA haps the mitochondrial haplotypes identified in each population (see also Figure 3). Published haplotypes from NW Iberian *S. salamandra* (Beukema, Nicieza, Lourenço, & Velo-Antón, 2016; Velo-Antón et al., 2007) and *L. boscai* samples (Teixeira et al., 2015) found out of the island-mainland system are not represented in this table

Species	Group	Pop ID	Locality	Lat.	Long.	*N*	P_A	H_O	H_E	A_R	*R*	N-mtDNA	mtDNA haps
S. salamandra	IS	ONS	Ons	42,38	−8,93	31	8	0.66	0.70	5.25	0.29	4	9, 10
S. salamandra	IS	SM	San Martiño*	42,20	−8,91	32	0	0.56	0.57	3.70	0.49	4	10
S. salamandra	H-IS	GRO	Grove	42,47	−8,89	36	4	0.56	0.67	4.57	0.27	1	1
S. salamandra	MAIN	HOM	Cabo Home	42,25	−8,86	28	3	0.64	0.71	5.34	0.22	3	13
S. salamandra	H-IS	MFE	Monteferro*	42,15	−8,84	31	2	0.74	0.74	5.10	0.24	2	13
S. salamandra	MAIN	CUR	Curotiña	42,67	−8,98	7	0	0.75	0.71	—	—	1	10
S. salamandra	MAIN	XUR	San Xurxo	42,52	−8,51	20	0	0.77	0.85	6.63	0.03	3	1, 5
S. salamandra	MAIN	CAS	Castrove	42,46	−8,71	25	2	0.75	0.80	6.99	0.08	2	1
S. salamandra	MAIN	LOU	Lourizán*	42,41	−8,67	32	1	0.85	0.86	7.75	0.01	2	1
S. salamandra	MAIN	COT	Cotorredondo*	42,36	−8,68	25	1	0.86	0.86	8.21	−0.01	2	1
S. salamandra	MAIN	COI	Coiro	42,30	−8,77	30	2	0.77	0.82	7.30	0.04	2	1
S. salamandra	MAIN	NER	Nerga*	42,26	−8,82	6	0	0.77	0.88	—	—	1	13
S. salamandra	MAIN	VIG	Vigo*	42,23	−8,71	6	0	0.83	0.80	—	—	4	1, 7
S. salamandra	MAIN	BAR	Baredo*	42,10	−8,87	34	2	0.80	0.85	7.17	0.04	1	14
S. salamandra	MAIN	SAR	Saramagal*	42,13	−8,69	11	2	0.78	0.85	7.12	0.04	2	1
S. salamandra	MAIN	ALO	Aloia	42,08	−8,69	15	5	0.84	0.83	7.08	0.07	2	1, 7
Total						369						36	
L. boscai	IS	SAL	Sálvora*	42,47	−9,01	11	0	0.60	0.67	4.29	0.29	3	12
L. boscai	IS	ONS	Ons	42,38	−8,93	38	0	0.58	0.60	3.54	0.39	3	19
L. boscai	H-IS	GRO	Grove	42,47	−8,89	11	1	0.63	0.79	4.98	−0.02	1	NA
L. boscai	MAIN	HOM	Cabo Home	42,25	−8,86	15	0	0.65	0.74	4.66	0.13	3	1
L. boscai	MAIN	UDR	Cabo Udra*	42,33	−8,82	15	0	0.66	0.72	4.42	0.17	2	1, 13
L. boscai	MAIN	NOI	Noia*	42,73	−8,86	32	4	0.62	0.77	6.03	0.10	NA	NA
L. boscai	MAIN	CUR	Curotiña	42,31	−8,59	4	1	0.75	0.80	—	—	6	14, 16, 21, 22
L. boscai	MAIN	XUR	San Xurxo	42,52	−8,51	6	2	0.75	0.83	—	—	2	1, 13
L. boscai	MAIN	SIS	Sisán*	42,49	−8,80	10	1	0.67	0.76	4.79	0.11	NA	13, 17
L. boscai	MAIN	CAS	Monte Castrove	42,46	−8,71	18	1	0.65	0.76	4.82	0.08	4	8, 13, 20
L. boscai	MAIN	PON	Pontevedra*	42,45	−8,61	3	0	0.71	0.81	—	—	2	9, 11
L. boscai	MAIN	COI	Coiro	42,30	−8,77	15	2	0.70	0.75	4.68	0.10	2	15
L. boscai	MAIN	ARC	Arcade*	42,36	−8,59	15	1	0.79	0.80	5.01	0.05	2	1
L. boscai	MAIN	RED	Redondela*	42,30	−8,59	5	0	0.83	0.79	—	—	2	1, 7
L. boscai	MAIN	COR	Coruxo*	42,17	−8,78	3	1	0.68	0.69	—	—	1	1
L. boscai	MAIN	ALO	Monte Aloia	42,08	−8,69	21	3	0.70	0.78	5.09	0.07	2	13, 18
Total						222						35	

2.4 | Population genetic analyses

Microsatellite analyses were performed for the entire sample data sets of both species. We tested for departures from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium (LE) in GENEPOP 4.2 (Rousset, 2008). The false discovery rate (Benjamini & Hochberg, 1995) was applied to correct p-values from HWE and LE multiple exact tests. Only *L. boscai* locus Ltb12 showed consistent deviations from HWE and LE and thus was excluded from downstream analyses. Additionally, there was no evidence for null alleles as revealed by INEST 2.0 (Chybicki & Burczyk, 2009).

Observed (H_O) and expected heterozygosity (H_E), number of private alleles (P_A) and population average relatedness (*R*) were calculated in GENALEX 6.5 (Peakall & Smouse, 2012). Allelic richness (A_R) was calculated in the R 3.4.0 statistical platform (R Development Core Team, 2017) with the R package "diveRsity" (Keenan,

McGinnity, Cross, Crozier, & Prodöhl, 2013). To avoid bias in diversity estimates, R and A_R were calculated only in localities containing ten or more samples.

2.5 | Genetic structure and gene flow analyses

We calculated pairwise F_{ST} values and respective 95% confidence intervals (CIs) in the R package "diveRsity." Pairwise F_{ST} values were acknowledged as significant when 95% CIs did not overlap with zero.

We used the Bayesian clustering algorithm implemented in STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) to assess genetic structure patterns. We set the admixture model with correlated allele frequencies for each analysis. No informative priors regarding sampling location were set. Ten independent runs for a number of clusters (K) ranging between 1 and 20 were computed (considering potential substructure within populations). Each run comprised 5×10^5 Markov chain Monte Carlo (MCMC) iterations following a burn-in period of 5×10^4 iterations. Input parameters were equal between the studied species. STRUCTURE output was summarized and graphically illustrated using the main pipeline implemented in CLUMPAK with default advanced options (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015). The most supported K was the one exhibiting the largest ΔK value, as estimated in CLUMPAK (Evanno, Regnaut, & Goudet, 2005).

To visualize geographical patterns of genetic diversity and structure in both species, we used ARCGIS 10.1 (ESRI, Redland, CA, USA). A_R and pairwise F_{ST} values were spatially interpolated using the kriging method implemented in "Geostatistical Analyst" extension. A principal component analysis (PCA) was used to summarize the resulting interpolated surfaces obtained from pairwise F_{ST} values by sampled locality.

Lissotriton boscai from Sálvora Island revealed genetic admixture patterns with the mainland (see Results). Following these results, we estimated also contemporary gene flow (within the last few generations) between islands and mainland localities in both species to help us better understand contemporary genetic patterns. We used a Bayesian approach implemented in BAYESASS 3.0 (Wilson & Rannala, 2003), and detailed methodological procedures are described in Appendix S1.

2.6 | Colonization history

Colonization history of both species was investigated using the microsatellite data sets under an ABC statistical framework implemented in DIYABC 2.1 (Cornuet et al., 2014). For *L. boscai*, six loci were used in this analysis as Ltb17 was discarded due to an irregular repeat length motif. To avoid overly complex demographic scenarios, we used only a subset of sampled localities based on STRUCTURE's results and their geographical location. For *S. salamandra*, we included both insular populations (Ons and San Martiño), the nearest coastal sampled localities that may have been a source of immigrants to the islands (Grove, Cabo Home and Monteferro) and a deme representative of the genetic composition of the mainland comprised by remaining continental populations (n = 211). For *L. boscai*, a total of three demes were included: both insular populations (Ons and Sálvora) and a mainland deme constituted by all mainland localities (n = 173). We assumed a generation time of 4 years for *S. salamandra* (Alcobendas & Castanet, 2000) and 3 years for *L. boscai* (Caetano & Leclair, 1999).

We performed trial runs with varying prior values to check for misspecifications of our models (see Appendix S1). In the final runs, for each assessed population, we set a wide prior 10–10,000 (10–20,000 for mainland demes) for the contemporary *Ne*. The priors for dates of population divergence were set to a maximum of 5,000 generations to encompass changes in the sea level during the Holocene (see Table S2.1 in Appendix S2). We used a generalized stepwise mutation model following a gamma distribution and with default mean rate values. Five summary statistics were chosen to characterize genetic variation among and between populations in simulated data sets and compared with the ones from the observed data set: (1) one-sample mean number of alleles, (2) one-sample mean of genetic diversity, (3) two-sample mean number of alleles, (4) two-sample mean of genetic diversity and (5) pairwise F_{ST}.

A recent study found that DIYABC has poor performance in recovering the true model when more than three complex candidate demographic scenarios are tested (Cabrera & Palsbøll, 2017). Accordingly, we devised a total of three and two scenarios for *S. salamandra* and *L. boscai* respectively (Figure 2b). These scenarios contrast vicariance versus colonization via dispersal as putative mechanisms responsible for the origin of insular populations. As no evidence for gene flow between mainland and insular populations of *L. boscai* was observed (see Results), we simplified models by not accounting for it. Moreover, in addition to STRUCTURE's results and the geographical position of sampled localities, we used the bathymetric profile of the region and the pattern of historical sea level rises derived from geological data (Dias et al., 2000) to define tree topologies. The scenarios tested were vicariance and colonization via dispersal.

1. Vicariance (VIC_Ssal and VIC_Lbos)—Both scenarios assume that insular populations originated by a vicariant event promoted by a sea level rise during the Holocene. In *S. salamandra*, scenario VIC_Ssal assumes an early split of Ons and San Martiño populations from the mainland deme at T_{H0}. When the sea level reached its maximum (T_{HM}), the coastal populations of Grove, Cabo Home and Monteferro diverged from the mainland. Grove and Monteferro showed high genetic differentiation levels in the present study (see Results; see also Velo-Antón et al., 2012). Both are located in small peninsulas, which together with their genetic patterns and available geological evidence (Dias et al., 2000; see also Discussion), led Velo-Antón et al. (2012) to suggest that both may have been islands temporarily. Given that our primary objective was to assess colonization history of Ons and San Martiño, for simplicity, we assumed concurrent divergence of these coastal populations. In *L. boscai*, scenario VIC_Lbos depicts an early split of Ons at T_{H0}. Because Sálvora is separated from the mainland by a lower bathymetric profile, it was isolated later at T_{H1} and diverged from the mainland more recently.

2. Colonization via dispersal (CVD1_Ssal, CVD2_Ssal and CVD_Lbos) —These scenarios assume a recent colonization of the islands via dispersal of few immigrants from continental populations. These models assume a very low initial *Ne* of insular populations (we constrained the *Ne* prior to a maximum value of 50) at the time of colonization to simulate a strong population bottleneck caused by founder events (Table S2.1 in Appendix S2; Figure 2b). In *S. salamandra*, following divergence of coastal populations (Grove, Cabo Home and Monteferro) at T_{HM}, Ons and San Martiño were colonized by a few immigrants from Grove and Cabo Home, respectively (CVD1_Ssal), at T_{C0}. Following the establishment of both islands, the *Ne* substantially increased at T_{C1}. As Monteferro is close to San Martiño Island, it could have also acted as a source of immigrants. Thus, an additional scenario modelling colonization from Monteferro to San Martiño was tested (CVD2_Ssal). In *L. boscai*, Ons and Sálvora are assumed to be colonized from the mainland at T_{C0}, followed by an increase in *Ne* at T_{C1} (CVD_Lbos).

We calculated the posterior probabilities (PP) and respective 95% CIs of the competing scenarios. The demographic scenario exhibiting the highest PP and non-overlapping 95% CIs with other scenarios was considered as the most supported scenario. Type-I (probability of rejecting the selected scenario when it is true; α) and type-II errors (probability of selecting the scenario when it is false; β) were calculated to assess confidence in scenario choice by generating 200 pseudo-observed data sets (PODs; 175 PODs were generated for *L. boscai* due to computational limitations). Posterior parameters were estimated and models checked. All methodological procedures performed in DIYABC are described in Appendix S1.

3 | RESULTS

3.1 | Phylogeographical analyses

In *S. salamandra*, both insular and two coastal populations (Home and Monteferro), together with three mainland populations, comprised a haplogroup that is widespread along NW Iberia, whereas the remaining mainland populations clustered in a slightly divergent haplogroup that is parapatrically distributed along this region. Of the 15 total haplotypes found, only two were in the insular populations (Figure 3a; Table 1): haplotype 10 is shared among insular and mainland populations, and haplotype 9 is endemic to Ons.

In *L. boscai*, samples grouped into two slightly divergent haplogroups (Figure 3b; Table 1) with two of the 22 total haplotypes observed found only in the insular populations. Haplotypes 12 and 19 are endemic to Ons and Sálvora, respectively, and both derive from the most common haplotypes occurring in nearby coastal areas (e.g. Cabo Home) and in mainland populations.

3.2 | Population genetic analyses

Both species presented high mean diversity levels, though diversity was higher in *S. salamandra* (H_O = 0.746; A_R = 6.32) than in *L. boscai* (H_O = 0.685; A_R = 4.76) (Table 1). In the former species, mainland populations (mean H_O = 0.785 and A_R = 7.06) exhibited higher diversity levels compared with populations located on historical (H_O = 0.651; A_R = 4.84) and contemporary islands (H_O = 0.608; A_R = 4.48) (Figure 4a). This trend was not as evident in *L. boscai* populations (Figure 4b). However, the insular populations of *S. salamandra* on San Martiño (H_O = 0.561; A_R = 3.70; R = 0.490) and *L. boscai* on Ons (H_O = 0.581; A_R = 3.54; R = 0.390) exhibited the lowest genetic variation and the highest relatedness. P_A was low overall, except for the *S. salamandra* population on Ons (P_A = 8).

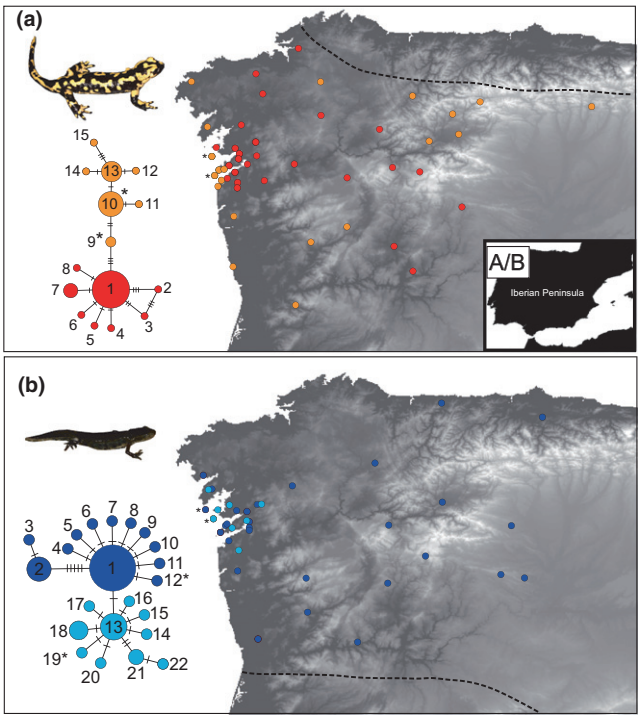

FIGURE 3 mtDNA haplotype networks and geographical distribution of main haplogroups identified in north-western Spain for *Salamandra salamandra* (a) and *Lissotriton boscai* (b). Asterisks in the haplotype networks denote the haplotypes found in insular populations. Black dashed lines indicate the contact zones with other subspecies or lineages

3.3 | Genetic structure and gene flow analyses

Average pairwise genetic differentiation was moderate in both *S. salamandra* (mean population pairwise F_{ST} = 0.089; Table S2.2 in Appendix S2) and *L. boscai* (mean population pairwise F_{ST} = 0.075; Table S2.3 in Appendix S2). Among both species, the highest values of differentiation were estimated for insular populations (*S. salamandra*, Ons, F_{ST} = 0.165; San Martiño, F_{ST} = 0.227; *L. boscai*, Ons, F_{ST} = 0.205; Sálvora, F_{ST} = 0.124). *Salamandra salamandra* populations located on the two putative historical islands also showed higher values of genetic differentiation (Grove, F_{ST} = 0.133; Monteferro, F_{ST} = 0.126). Notwithstanding, populations in Cabo Home (F_{ST} = 0.114) and Curotiña (F_{ST} = 0.125) also presented high overall genetic differentiation, though the latter has a very low sample size

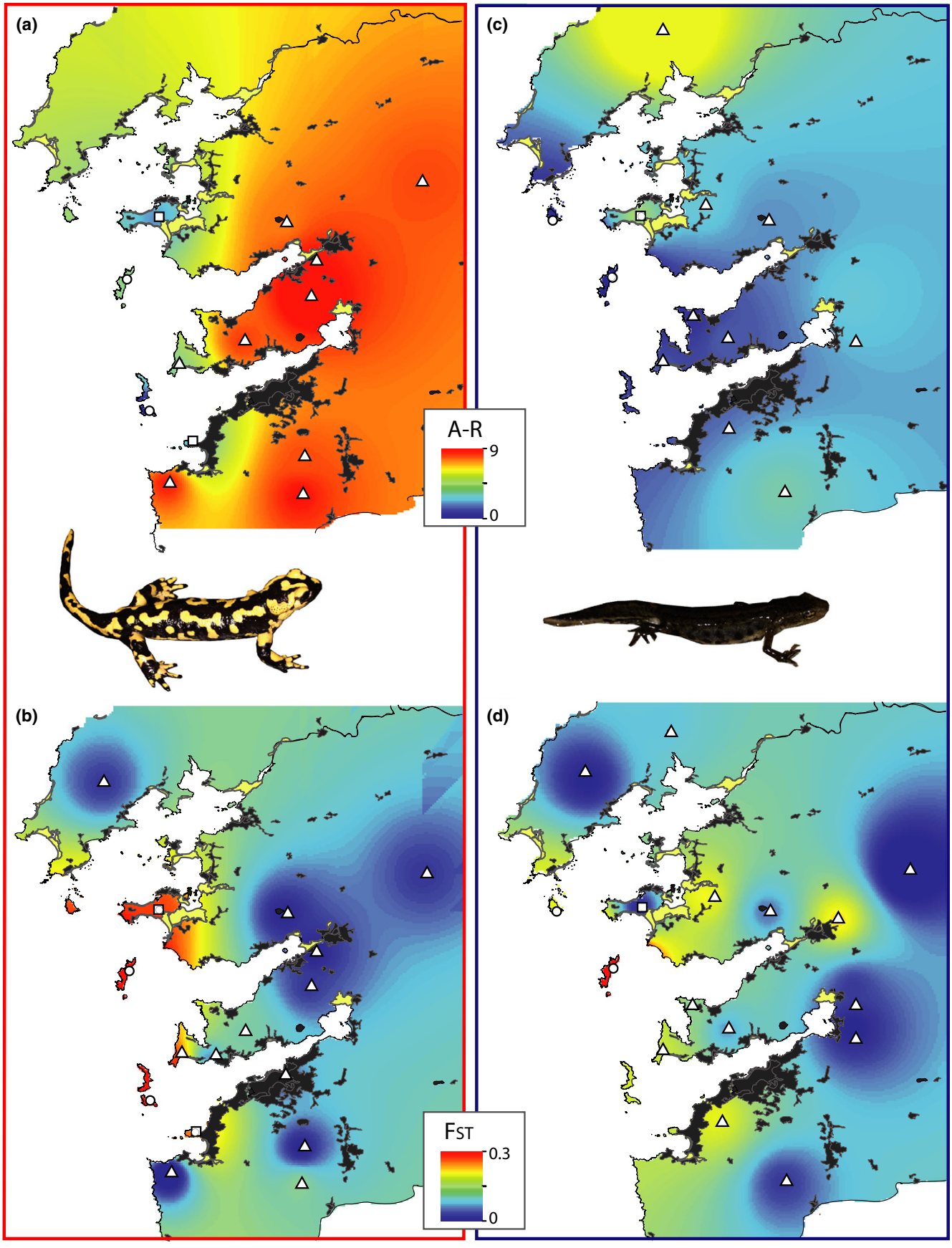

FIGURE 4 Spatial interpolation of A_R and pairwise F_{ST} between populations of *Salamandra salamandra* (panels a and c) and *Lissotriton boscai* (panels b and d) in north-western Spain. Circles, squares and triangles denote sampled localities in islands, historical islands and mainland respectively

(remaining mainland populations had a mean $F_{ST} < 0.098$) (Figure 4c). In *L. boscai*, all mainland populations showed similar values (Table S2.3 in Appendix S2; Figure 4d).

For *S. salamandra*, STRUCTURE identified the optimal scenario of $K = 6$ (Figure 5a). Five populations are geographically well delimited, namely the two insular populations and three coastal populations (Cabo Home and the putative historical insular populations of Grove and Monteferro). Mainland populations comprised a distinct genetic cluster, showing a marked pattern of genetic admixture with Cabo Home. For *L. boscai*, only the insular population of Ons showed a strong genetic structure. Sálvora and mainland populations clustered into two genetic groups that showed extensive genetic admixture for an optimal scenario of $K = 3$ (Figure 5b). BAYESASS revealed no gene flow between the islands and the mainland in both species (Table S2.4 in Appendix S2).

3.4 | Colonization history

A pre-evaluation of the chosen priors revealed a good fit between simulated and observed data sets for both species (Fig. S3.1 in Appendix S3). For both species, DIYABC showed unequivocal support for scenarios modelling vicariance as the main process originating insular populations (VIC_Ssal, PP = 0.98, 95% CIs: 0.97–0.98; VIC_Lbos, PP = 0.84, 95% CIs: 0.83–0.84) (see Fig. S3.2 in Appendix S3). Type-I (Vic_Ssal, $\alpha = 0.02$; Vic_Lbos, $\alpha = 0.04$) and type-II errors (Vic_Ssal, $\beta = 0.06$; Vic_Lbos, $\beta = 0.03$) were low for the most supported scenarios for both species, demonstrating high confidence in scenario choice.

The estimated mean posterior parameters of *Ne* for *S. salamandra* populations, under scenario VIC_Ssal, showed that San Martiño had the lowest *Ne* (*Ne* = 1530, 95% CIs: 696–2820), while Ons exhibited larger *Ne* estimates compared with coastal populations (*Ne* = 4840, 95% CIs: 2030–8750, Fig. S3.3a in Appendix S3). The estimated mean date of divergence between insular populations and the mainland (T_{H0}) was 10.9 kya (95% CIs: 3.4–19.3 kya), followed by a more recent split of coastal populations (T_{HM} = 3.8 kya, 95% CIs: 1.2–10 kya). For *L. boscai* populations, Sálvora (*Ne* = 2370, 95% CIs: 466–6930) showed a higher *Ne* than Ons (*Ne* = 1760, 95% CIs: 660–3580) (Fig. S3.3b in Appendix S3). The insular population of Ons diverged from the mainland (T_{H0} = 8.0 kya, 95% CIs: 2.0–14.6 kya) earlier than Sálvora (T_{H1} = 2.9 kya, 95% CIs: 0.4–8.8 kya). All posterior parameter estimates are shown in Table S2.5 in Appendix S2.

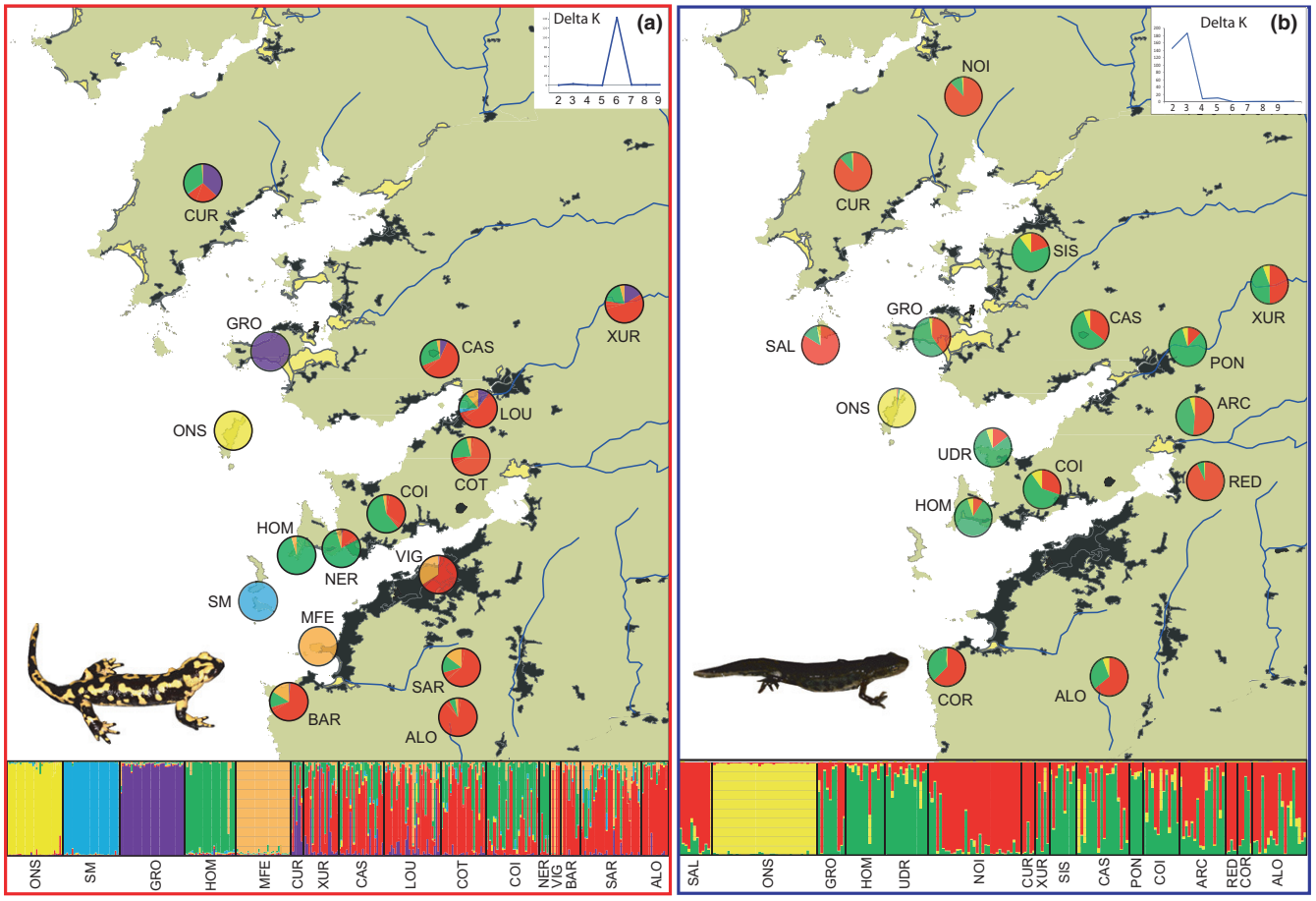

FIGURE 5 Map of north-western Spain containing pie charts highlighting the population cluster membership obtained in STRUCTURE for (a) *Salamandra salamandra* and (b) *Lissotriton boscai*. In the top-right corner of each panel, plots of the ΔK parameter are shown to highlight the most supported *K*. STRUCTURE barplots are displayed below the map of the study area

Under this method, parameter estimations were precise, as shown by the small values of the relative median absolute deviation (Table S2.5 in Appendix S2). Model checking revealed that simulated data sets obtained from the parameter posterior distributions closely resemble the observed data in both species (Fig. S3.4 in Appendix S3).

4 | DISCUSSION

4.1 | Patterns of genetic variation and *Ne* in mainland and insular populations

Both species' ranges were affected by contractions and expansions in response to cyclic climatic oscillations during the Pleistocene. On the expansion front, genetic variation is usually lower than core populations due to surfing allele processes along the expansion wave (Excoffier, Foll, & Petit, 2009), with time being key for the arrival of new alleles to this front and boost genetic diversity. This could explain the lower genetic diversity values in mainland populations of *L. boscai* compared to those observed in *S. salamandra*. The former must arrived much more recently to the NW Iberian Peninsula than the latter (García-París et al., 2003; Teixeira et al., 2015).

Salamandra salamandra populations inhabiting the putative historical islands (Grove and Monteferro) exhibited lower genetic diversity and a stronger genetic structure than the other mainland populations. The estimated date of divergence of these populations from the mainland (3.7 kya) is congruent with the period in which the sea level reached its current level (3.5 kya; Dias et al., 2000), further supporting the hypothesis that these two localities were islands in the recent past. Furthermore, Velo-Antón et al. (2012) previously suggested that Cabo Home (referred to as Melide in that study) may have been a historical island due to the strong genetic structure and lower diversity observed in *S. salamandra*. However, by sampling more mainland localities near Cabo Home, we show that the extent of genetic admixed observed with Cabo Home is inconsistent with it being a historical island (Figure 5). Conversely, the Grove population of *L. boscai* presents similar genetic variation patterns as other mainland populations. Two non-exclusive factors may explain these patterns. First, higher fecundity rates observed in *L. boscai* females might have helped maintain a larger *Ne* during isolation, reducing the detrimental genetic effects of drift (Ellegren & Galtier, 2016; Wang et al., 2014). Second, putative gene flow between Grove and mainland populations may have also alleviated the effects of genetic isolation. Unlike *S. salamandra*, the aquatic life style of *L. boscai* may have facilitated the dispersal of a considerable number of individuals to Grove, especially given the small geographical distance between areas. The short-term migration rates from the mainland deme to Grove were not significant though, suggesting that this population may be isolated from the other continental populations, but additional populations sampled nearby Grove, as well as, increasing the number of loci are needed to clarify this issue.

Consistent with other studies focusing on land bridge populations of amphibians (e.g. Bell et al., 2012; Bessa-Silva et al., 2016; Wang et al., 2014), our study shows reduced genetic diversity and substantially higher genetic differentiation and relatedness in insular populations compared with their mainland counterparts, emphasizing the role of drift as a powerful driver of contemporary genetic variation (Ellegren & Galtier, 2016). Remarkably, the insular populations exhibit lower diversity levels than smaller sized ($Ne < 100$) urban populations of *S. salamandra*, most of which have been isolated for the last 100 generations (Lourenço, Álvarez, Wang, & Velo-Antón, 2017). This finding suggests that long-term isolation might have enhanced the deleterious effects of drift in these insular populations (Hurston et al., 2009; Wang et al., 2014).

For *S. salamandra*, the DIYABC analysis revealed a very large *Ne* on Ons, comparable to *Ne* estimates for coastal populations, which is in agreement with field observations (Velo-Antón & Cordero-Rivera, 2017). The larger size of Ons, and the suitable habitats found throughout the island (i.e. *Ulex* spp. shrubs), may have contributed to the high *Ne* and allelic diversity levels (Ellegren & Galtier, 2016; Hurston et al., 2009). *Salamandra salamandra* population of San Martiño is much smaller than that of Ons, likely due to less favourable habitats (*Eucalyptus* spp. plantations) and higher predation pressure (Velo-Antón & Cordero-Rivera, 2017), which may explain why this population is less diverse. Moreover, despite being the less fecund species, the insular population of *S. salamandra* on Ons presented a higher *Ne* than the insular populations of *L. boscai*. However, the demographic estimates in our ABC analysis, with their wide 95% CIs, may not accurately reflect the *Ne* of *L. boscai* insular populations due to insufficient genetic data.

4.2 | Vicariance versus colonization via oversea dispersal

The low mtDNA divergence and haplotype sharing observed between insular and mainland populations indicate a recent shared evolutionary history in this island-mainland population system in both urodele species. Yet, the endemic mtDNA haplotypes found in insular populations of *L. boscai* and one insular population of *S. salamandra* do not support a scenario of colonization and/or high marine migration rates, suggesting that vicariance was the main driver of the observed genetic differences between insular and mainland populations.

Our ABC framework also supports a scenario of vicariance for both species, corroborating other studies highlighting its influence on contemporary genetic variation in amphibian land bridge populations (e.g. Bell et al., 2012; Bessa-Silva et al., 2016; Duryea, Zamudio, & Brasileiro, 2015; Wang et al., 2014). Interestingly, the mean date of divergence of insular populations from the mainland deme (10.9 kya for insular populations of *S. salamandra*; 8.0 kya for *L. boscai* on Ons) coincides with the estimated period in which coastal lowlands flooded due to increasing seawater levels (ca. 3.5–13 kya; Dias et al., 2000). DIYABC also estimated a very recent divergence of the Sálvora population (2.9 kya), suggesting that *L. boscai* on this island became isolated only after the sea level reached its maximum (Dias et al., 2000). However, these divergence estimates should be interpreted with caution, particularly for *L. boscai*, as Cabrera and Palsbøll (2017) found a high level of inaccuracy in estimates based on insufficient genetic data.

4.3 | Is there evidence for gene flow between the continent and islands?

Our analyses revealed a lack of short-term gene flow between the islands and the mainland for both species. However, the *L. boscai* population of Sálvora revealed admixture patterns with nearby mainland populations (e.g. Noia and Curotiña), implying potential contemporary gene flow from the mainland. We argue that these admixture patterns rather reflect incomplete lineage sorting caused by a complex interplay between bathymetry (i.e. later formation of this island), availability of suitable habitats and the *Ne* in Sálvora.

The bathymetric topography of the sea floor is an important determinant of the time since isolation of land bridge populations. Land bridge islands separated from the mainland by a deeper bathymetric depression putatively experienced an earlier vicariant process and, consequently, an earlier interruption of gene flow with the mainland (e.g. beetles in the Aegean archipelago, Papadopoulou & Knowles, 2015). The island of Sálvora is geographically connected to the mainland by a shallower bathymetric topography, and there are several small islets in between. This indicates that Sálvora was isolated by the ocean later than Ons and San Martiño (as corroborated by DIYABC). Therefore, gene flow might have been maintained for a longer period in a stepping-stone scenario, probably preventing the emergence of deep genetic structure. Additionally, Ons is much larger than Sálvora, and thus, could harbour a greater availability of resources, hypothetically having better conditions that lead to a larger population size and higher levels of genetic diversity (Hurston et al., 2009; Whittaker & Fernández-Palacios, 2007). However, we observed the opposite pattern, with the population of Sálvora displaying a much larger *Ne* than Ons. Unlike Ons, Sálvora presents a wide array of suitable aquatic habitats (e.g. ponds) to provide food and ideal conditions for reproduction in *L. boscai* (GVA, personal observation). This fact, coupled with the high fecundity rate of *L. boscai* females, may contribute to maintain a large *Ne* and high levels of diversity (Bessa-Silva et al., 2016; Leffler et al., 2012; Romiguier et al., 2014), and may also alleviate the divergent effects of drift under genetic isolation. Despite the role of the above factors in shaping contemporary genetic variation in Sálvora and the strong northward flowing marine currents in the studied region hindering overseas dispersal (Teles-Machado, Peliz, McWilliams, Couvelard, & Ambar, 2016), a small portion of individuals may indeed reach the islands and reproduce.

5 | CONCLUSIONS

Land bridge populations of both species exhibited reduced genetic diversity and increased genetic structure, demonstrating the role of long-term genetic isolation in driving contemporary genetic variation. Furthermore, the migration-drift equilibrium throughout the evolutionary history of insular populations of amphibians may also be influenced by the interplay of intrinsic (life history traits, such as tolerance to salinity) and extrinsic factors (e.g. island size, bathymetry, distance to mainland). The genetic patterns observed in Sálvora clearly illustrate this complexity. Finally, the increasing availability of genomic tools will enable researchers to focus on adaptive genetic variation, helping disentangle the contribution of drift and selection on genetic variation and improving our knowledge of the interaction between selection and drift in recently formed islands.

ACKNOWLEDGEMENTS

We thank Adolfo Cordero and Maria Casal for their help during fieldwork, and the employees of the National Park for facilitating our trips to the islands. Fernando Martínez-Freiría helped with spatial interpolation analysis. This work was funded by FEDER funds through the Operational Programme for Competitiveness Factors—COMPETE—and by National Funds through FCT—Foundation for Science and Technology—under the PTDC/BIA-EVF/3036/2012, POCI-01-0145-FEDER-006821 and FCOMP- 01-0124-FEDER-028325. GVA, AL and FS are supported by the Foundation for Science and Technology (IF/01425/2014, PD/BD/106060/2015 and UID/BIA/50027/2013, respectively). Fieldwork for obtaining tissue samples was done with the corresponding permits from the National Park and regional administration (Xunta de Galicia, Ref. 1653/2009, 014/2011, 546/2012, 027/2013).

DATA ACCESSIBILITY

GenBank accession numbers are the following: MG640339-MG640354 (*NAD4*; *L. boscai*), MG640355-MG640361 (cyt *b*; *S. salamandra*). Data sets of microsatellite genotypes for *Salamandra salamandra* and *Lissotriton boscai* can be found in Appendix S2.6 and S2.7 respectively.

ORCID

Guillermo Velo-Antón http://orcid.org/0000-0002-9483-5695

REFERENCES

Alcobendas, M., & Castanet, J. (2000). Bone growth plasticity among populations of *Salamandra salamandra*: Interactions between internal and external factors. *Herpetologica*, *56*, 14–26.

Arévalo, E., Davis, S. K., & Sites, J. W. Jr. (1994). Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Systematic Biology*, *43*, 387–418.

Bell, R., Brasileiro, C., Haddad, C. F. B., & Zamudio, K. R. (2012). Evolutionary history of *Scinax* treefrogs on land-bridge islands in southeastern. *Journal of Biogeography*, *39*, 1733–1742.

Bell, R. C., Drewes, R. C., & Zamudio, K. R. (2015). Reed frog diversification in the Gulf of Guinea: Overseas dispersal, the progression rule, and in situ speciation. *Evolution*, *69*, 904–915.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B*, *57*, 289–300.

Bessa-Silva, A. R., Vallinoto, M., Sodré, D., Cunha, D. B., Hadad, D., Asp, N. E., … Sequeira, F. (2016). Patterns of genetic variability in island

populations of the cane toad (*Rhinella marina*) from the mouth of the Amazon. *PLoS ONE*, *11*, e0152492.

Beukema, W., Nicieza, A. G., Lourenço, A., & Velo-Antón, G. (2016). Colour polymorphism in *Salamandra salamandra* (Amphibia: Urodela), revealed by a lack of genetic and environmental differentiation between distinct phenotypes. *Journal of Zoological Systematics and Evolutionary Research*, *54*, 127–136.

Cabrera, A. A., & Palsbøll, P. J. (2017). Inferring past demographic changes from contemporary genetic data: A simulation-based evaluation of the ABC methods implemented in DIYABC. *Molecular Ecology Resources*, *17*, 94–110.

Caetano, M. H., & Leclair, R. (1999). Comparative phenology and demography of *Triturus boscai* from Portugal. *Journal of Herpetology*, *33*, 192–202.

Chybicki, I. J., & Burczyk, J. (2009). Simultaneous estimation of null alleles and inbreeding coefficients. *The Journal of Heredity*, *100*, 106–113.

Clement, M., Posada, D. C. K. A., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1659.

Cornuet, J. M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., … Estoup, A. (2014). DIYABC v2.0: A software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism. DNA sequence and microsatellite data. *Bioinformatics*, *30*, 1187–1189.

Dias, J. M. A., Boski, T., Rodrigues, A., & Magalhães, F. (2000). Coast line evolution in Portugal since the Last Glacial Maximum until present—A synthesis. *Marine Geology*, *170*, 177–186.

Díaz-Paniagua, C. (2002). Tritón ibérico—*Lissotriton boscai*. In: L. M. Carrascal & A. Salvador (Eds.), *Enciclopedia Virtual de los Vertebrados Españoles*. Madrid: Museo Nacional de Ciencias Naturales. http://www.vertebradosibericos.org/

Duellman, W., & Trueb, L. (1994). *Biology of amphibians*. Baltimore, MA: The Johns Hopkins University Press.

Duryea, M. C., Zamudio, K. R., & Brasileiro, C. A. (2015). Vicariance and marine migration in continental island populations of a frog endemic to the Atlantic Coastal forest. *Heredity*, *115*, 225–234.

Ellegren, H., & Galtier, N. (2016). Determinants of genetic diversity. *Nature Reviews Genetics*, *17*, 422–433.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*, 2611–2620.

Excoffier, L., Foll, M., & Petit, R. J. (2009). Genetic consequences of range expansions. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 481–501.

Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? *Heredity*, *78*, 311–327.

Funk, W. C., Lovich, R. E., Hohenlohe, P. A., Hofman, C. A., Morrison, S. A., Sillett, T. S., … Andelt, W. F. (2016). Adaptive divergence despite strong genetic drift: Genomic analysis of the evolutionary mechanisms causing genetic differentiation in the island fox (*Urocyon littoralis*). *Molecular Ecology*, *25*, 2176–2194.

García-París, M., Alcobendas, M., Buckley, D., & Wake, D. B. (2003). Dispersal of viviparity across contact zones in Iberian populations of fire salamanders (*Salamandra*) inferred from discordance of genetic and morphological traits. *Evolution*, *57*, 129–143.

Gomez-Mestre, I., & Tejedo, M. (2003). Local adaptation of an anuran to osmotically stressful environments. *Evolution*, *57*, 1889–1899.

Hopkins, G. R., & Brodie, E. D. (2015). Occurrence of amphibians in saline habitats: A review and evolutionary perspective. *Herpetological Monographs*, *29*, 1–27.

Hopkins, G. R., Brodie, E. D., Neuman-Lee, L. A., Mohammadi, S., Brusch, G., Hopkins, Z., & French, S. (2016). Physiological responses to salinity vary with proximity to the ocean in a coastal amphibian. *Physiological and Biochemical Zoology*, *89*, 322–330.

Hurston, H., Voith, L., Bonanno, J., Foufopoulos, J., Pafilis, P., Valakos, E., & Anthony, N. (2009). Effects of fragmentation on genetic diversity in island populations of the Aegean wall lizard *Podarcis erhardii* (Lacertidae, Reptilia). *Molecular Phylogenetics and Evolution*, *52*, 395–405.

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). diveRsity: An R package for the estimation of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, *4*, 782–788.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*, 1179–1191.

Lambeck, K., Rouby, H., Purcell, A., Sun, Y., & Sambridge, M. (2014). Sea level and global ice volumes from the Last Glacial Maximum to the Holocene. *Proceedings of the National Academy of Sciences*, *111*, 15296–15303.

Leffler, E. M., Bullaughey, K., Matute, D. R., Meyer, W. K., Ségurel, L., Venkat, A., … Przeworski, M. (2012). Revisiting an old riddle: What determines genetic diversity levels within species? *PLoS Biology*, *10*, e1001388.

Lourenço, A., Álvarez, D., Wang, I. J., & Velo-Antón, G. (2017). Trapped within the city: Integrating demography, time since isolation and population-specific traits to assess the genetic effects of urbanization. *Molecular Ecology*, *26*, 1498–1514.

Martínez-Solano, I., & Lawson, R. (2009). Escape to Alcatraz: Evolutionary history of slender salamanders (*Batrachoseps*) on the islands of San Francisco Bay. *BMC Evolutionary Biology*, *9*, 38.

Martínez-Solano, I., Teixeira, J., Buckley, D., & García-París, M. (2006). Mitochondrial DNA phylogeography of *Lissotriton boscai* (Caudata, Salamandridae): Evidence for old, multiple refugia in an Iberian endemic. *Molecular Ecology*, *15*, 3375–3388.

Measey, G. J., Vences, M., Drewes, R. C., Chiari, Y., Melo, M., & Bourles, B. (2007). Freshwater paths across the ocean: Molecular phylogeny of the frog *Ptychadena newtoni* gives insights into amphibian colonization of oceanic islands. *Journal of Biogeography*, *34*, 7–20.

Papadopoulou, A., & Knowles, L. L. (2015). Species-specific responses to island connectivity cycles: Refined models for testing phylogeographic concordance across a Mediterranean Pleistocene Aggregate Island Complex. *Molecular Ecology*, *24*, 4252–4268.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics*, *28*, 2537–2539.

Peñalver-Alcázar, M., Martínez-Solano, I., Sequeira, F., & Aragón, P. (2017). Sex-dependent implications of primary productivity and conspecific density on geographic body size variation in a newt: Disentangling local, large scale and genetic factors. *Journal of Biogeography*, *44*, 2096–2108.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

R Development Core Team (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A., … Galtier, N. (2014). Comparative population genomics in animals uncovers the determinants of genetic diversity. *Nature*, *515*, 261–263.

Rousset, F. (2008). GENEPOP' 007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, *8*, 103–106.

Sequeira, F., Silva-Ferreira, A., & Lopes, S. (2012). Microsatellite markers for the Iberian endemic Bosca's newt, *Lissotriton boscai* (Caudata, Salamandridae). *Conservation Genetics Resources*, *4*, 715–717.

Steinfartz, S., Kuesters, D., & Tautz, D. (2004). Isolation and characterization of polymorphic tetranucleotide microsatellite loci in the fire

salamander *Salamandra salamandra* (Amphibia: Caudata). *Molecular Ecology Notes*, *4*, 626–628.

Teixeira, J., Martínez-Solano, I., Buckley, D., Tarroso, P., García-París, M., & Ferrand, N. (2015). Genealogy of the nuclear β-fibrinogen intron 7 in Lissotriton boscai (Caudata, Salamandridae): Concordance with mtDNA and implications for phylogeography and speciation. *Contributions to Zoology*, *84*, 193–215.

Teles-Machado, A., Peliz, Á., McWilliams, J. C., Couvelard, X., & Ambar, I. (2016). Circulation on the Northwestern Iberian Margin: Vertical structure and seasonality of the alongshore flows. *Progress in Oceanography*, *140*, 134–153.

Velo-Antón, G., & Cordero-Rivera, A. (2017). Ethological and phenotypic divergence in insular fire salamanders: Diurnal activity mediated by predation? *Acta Ethologica*, *20*, 243–253.

Velo-Antón, G., García-París, M., Galán, P., & Cordero Rivera, A. (2007). The evolution of viviparity in Holocene islands: Ecological adaptation versus phylogenetic descent along the transition from aquatic to terrestrial environments. *Journal of Zoological Systematics and Evolutionary Research*, *45*, 345–352.

Velo-Antón, G., Santos, X., Sanmartín-Villar, I., Cordero-Rivera, A., & Buckley, D. (2015). Intraspecific variation in clutch size and maternal investment in pueriparous and larviparous *Salamandra salamandra* females. *Evolutionary Ecology*, *29*, 185–204.

Velo-Antón, G., Zamudio, K. R., & Cordero-Rivera, A. (2012). Genetic drift and rapid evolution of viviparity in insular fire salamanders (*Salamandra salamandra*). *Heredity*, *108*, 410–418.

Vences, M., Vieites, D. R., Glaw, F., Brinkmann, H., Kosuch, J., Veith, M., & Meyer, A. (2003). Multiple overseas dispersal in amphibians. *Proceedings of the Royal Society B*, *270*, 2435–2442.

Wang, S., Zhu, W., Gao, X., Li, X., Yan, S., Liu, X., … Li, Y. (2014). Population size and time since island isolation determine genetic diversity loss in insular frog populations. *Molecular Ecology*, *23*, 637–648.

Whittaker, R. J., & Fernández-Palacios, J. M. (2007). *Island biogeography: Ecology, evolution, and conservation*. Oxford: Oxford University Press.

Wilson, G., & Rannala, B. (2003). Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, *163*, 1177–1191.

Yoichi, W., Jin, X.-F., Peng, C.-I., Tamaki, I., & Tomaru, N. (2017). Contrasting diversification history between insular and continental species of three-leaved azaleas (*Rhododendron* sect. *Brachycalyx*) in East Asia. *Journal of Biogeography*, *44*, 1065–1076.

Zhang, P., Papenfuss, T. J., Wake, M. H., Qu, L., & Wake, D. B. (2008). Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudate) inferred from complete mitochondrial genomes. *Molecular Phylogenetics and Evolution*, *49*, 586–597.

BIOSKETCH

André Lourenço is a PhD candidate at University of Porto, Portugal. He is interested in the eco-evolutionary implications of the evolution of viviparity in *Salamandra salamandra*.

Fernando Sequeira is an Associate Researcher at CIBIO-InBIO (University of Porto, Portugal) and an evolutionary biologist interested in population genetics, especially in understanding the processes of differentiation from population-level divergence to speciation.

David Buckley is an Associate Researcher at the MNCN-CSIC, and he is interested in understanding diversification, speciation and the evolution of novelties in animals.

Guillermo Velo-Antón is an Associate Researcher at CIBIO-InBIO (University of Porto, Portugal) and an evolutionary biologist studying how eco-evolutionary processes operate at different time-scales.

Author contributions: GVA conceived the ideas; GVA and FS collected the data; AL and GVA analysed the data; and AL led the writing with important contributions from all authors.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Lourenço A, Sequeira F, Buckley D, Velo-Antón G. Role of colonization history and species-specific traits on contemporary genetic variation of two salamander species in a Holocene island-mainland system. *J Biogeogr*. 2018;00:1–13. https://doi.org/10.1111/jbi.13192