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Isolation of lizard populations measured with molecular genetic data – *Podarcis guadarramae* in the Ria de Arosa archipelago

Raluca I. Băncilă[1,2,3], Jan W. Arntzen[1,*]

Abstract. Under a documented paleogeographic history of the Ria de Arosa archipelago, northwestern Spain, predictions can be made on the duration and strength of isolation of *Podarcis guadarramae* lizard populations that were 'captured' on the islands when sea-levels rose. We predict that genetic diversity: i) is lower on islands than on the mainland, ii) increases with island size, and iii) decreases with increasing distance from the mainland. We also predict that the population genetic structure across islands and the mainland conforms to a pattern of isolation by distance (iv). To test these hypotheses, we analysed a panel of nine highly polymorphic microsatellite loci. The first (i) hypothesis was corroborated. We discuss our findings in the light of the effects of habitat fragmentation and lizard conservation.

Keywords: genetic diversity, islands, isolation by distance, microsatellites, spatio-temporal isolation.

The Ria de Arosa is an inlet of the Atlantic Ocean at the north-western corner of the Iberian Peninsula. It is the largest (ca. 230 km^2), most irregularly shaped and most diversified of the four 'Rias Bajas' of Galicia. The rias have come into existence through a rise in sea-level towards the end of the last glacial period (Pannekoek, 1966; Bard et al., 1996). The inundation process started ca. 14 000 years BP (Pethick, 1984; Dias et al., 2000). Under this scenario, predictions can be made on the duration and strength of isolation of animal populations that were 'captured' on the islands. Although Arntzen and Sá-Sousa (2007) inferred one oversea dispersal event from the island Jidoiro Pedregoso to the neighbouring Jidoiro Arenoso, the off-shore Ria de Arosa lizard populations generally seem to be isolated from one another. The paleogeographic history of the Ria de Arosa therewith mimics the process of habitat fragmentation at a large spatio-temporal setting and makes a promising system to study the effect of population isolation over time and space.

The wall-lizard *Podarcis guadarramae* is commonly distributed over northwestern Spain, including the Ria de Arosa archipelago and the adjacent mainland (Arntzen and Sá-Sousa, 2007; Geniez et al., 2014). We test the hypotheses that: i) island populations retain lower genetic diversity than mainland populations as the result of a longer population isolation and limited gene flow, ii) small islands carry a lower genetic diversity than large islands because of differences in lizard population size, iii) genetic diversity decreases with increasing distance from the mainland because the time elapsed since isolation of remote island populations is longer than of those close to mainland and iv) the genetic structure of island populations conforms to a pattern of isolation-by-distance.

Tissue samples available for molecular genetic analysis were 101 *P. guadarramae*. The material was collected in July 2002 by M. S. Hoogmoed, J. M. Oliveira and the senior author from six islands in the Ria de Arosa archipelago (Coroso, Jidoiro Pedregroso, Noro, Rua, Salvora and Vionta) and three mainland localities adjacent to the Ria (fig. 1 and table 1, for exact locality information and for voucher material see Arntzen and Sá-Sousa, 2007). The surface area of the islands varies between 1.2 and 190 ha whereas the distances to the mainland between 0.22 km and 6.64 km (table 1).

All individuals were genotyped at nine microsatellite markers developed by Pinho et al. (2004). PCRs were run

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1 - Naturalis Biodiversity Center, P.O. Box 9517, 2300 RA Leiden, The Netherlands
2 - Institute of Speleology "Emil Racoviţă", 13 Septembrie Road, 050711, Bucharest, Romania
3 - Faculty of Natural Sciences, University Ovidius Constanţa, Blv. Mamaia 124, Constanţa, Romania
*Corresponding author; e-mail: pim.arntzen@naturalis.nl

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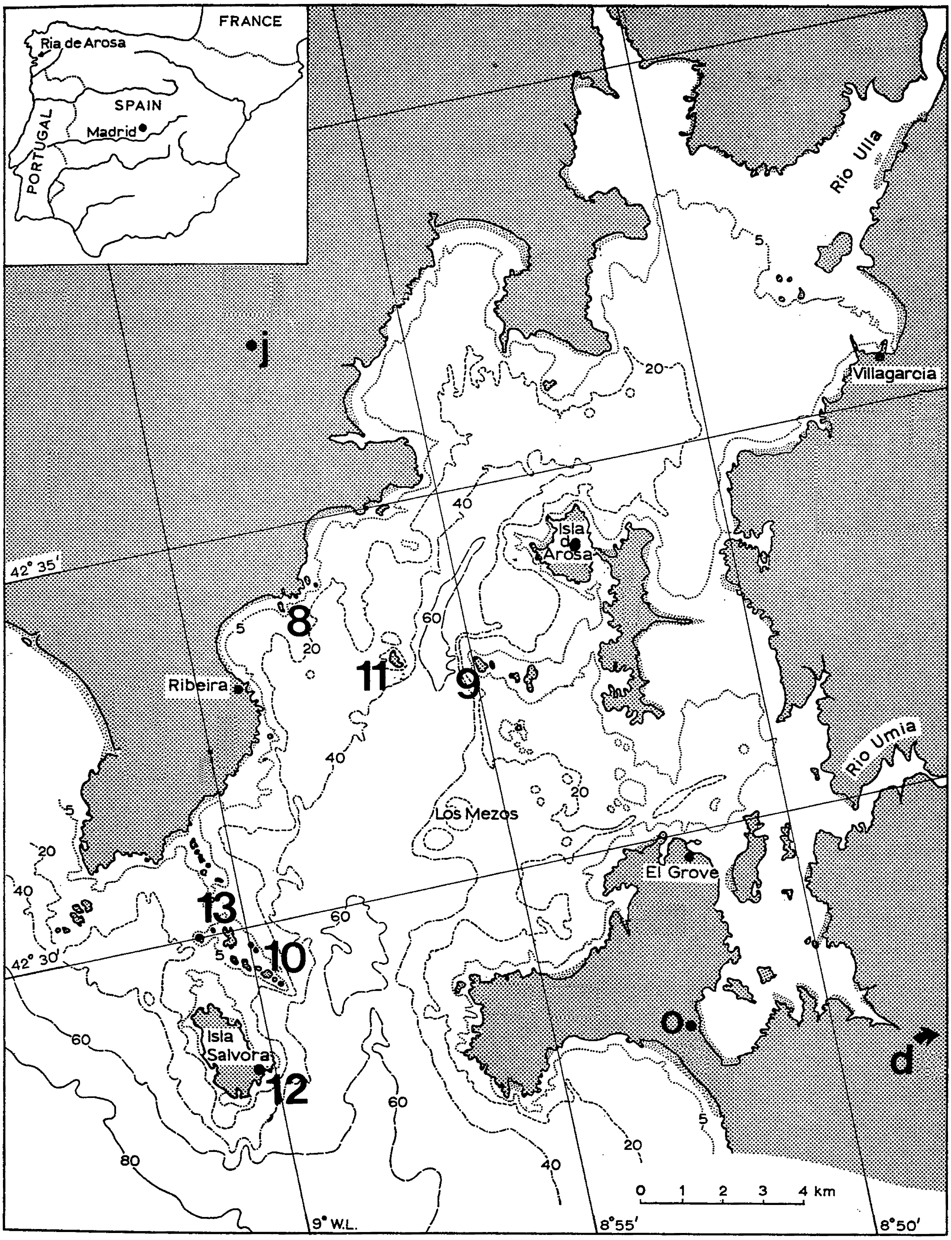

Figure 1. Situation map and bathymetry of the Ria de Arosa archipelago, Galicia, Spain, after Cadée (1968) and Arntzen and Sá-Sousa (2007). Island and mainland localities where *Podarcis guadarramae* was sampled are indicated by numbers and letters, as in table 1.

as described in Pinho et al. (2004). The amplification products were analysed by capillary gel electrophoresis on a MegaBACE 500 DNA Analysis System (Amersham Biosciences). Sizing of fragments was performed using the software MegaBACE Fragment Profiler 1.2. With FSTAT v. 2.9.3 (Goudet, 2001) we calculated allelic richness (A_r), observed heterozygosity (H_o), gene diversity (H_s) and inbreeding coefficient (F_{IS}). Deviation from Hardy-Weinberg equilibrium was assessed per locus using the exact probability test as implemented in GenePop 4.2 (Raymond and Rousset, 1995), with Markov chain parameters employing 10 000 dememorizations, 1000 batches and 10 000 iterations per batch and with application of the sequential Bonferroni correction for multiple comparisons (Rice, 1989). To test for linkage disequilibrium, we performed two-locus analyses of population subdivision (the D-stastistic of Ohta, 1982) with

Table 1. The sampled populations, map code, surface area of the islands (ha) and their distance to the mainland (km), the number of lizards sampled (N) and the genetic parameters of *Podarcis guadarramae* lizard populations in Ria de Arosa, Spain, based on nine microsatellite loci. A_r – allelic richness, H_o – observed heterozygosity, H_s – gene diversity and F_{IS} – inbreeding coefficient. For locality information and for voucher material see fig. 1 and the Appendix S1 in Arntzen and Sá-Sousa (2007).

Population	Map code	Area (ha)	Distance (km)	N	A_r	H_o	H_s	F_{IS}
Mostero da Armenteira – Mostero do Poio	d	–	–	7	4.17	0.81	0.85	0.04
Mirador de la Curotá	j	–	–	11	4.36	0.88	0.87	−0.01
Quarry in Coto de Caza San Martin	o	–	–	21	3.74	0.77	0.76	−0.02
Isla Coroso	8	1.20	0.22	7	3.17	0.75	0.71	−0.07
Isla Jidoiro Pedegroso	9	8.70	6.64	6	2.42	0.49	0.48	−0.01
Isla Noro	10	3.18	4.44	12	2.83	0.65	0.59	−0.10
Isla Rua	11	4.67	3.67	27	2.58	0.49	0.55	0.11
Isla Salvora	12	190	3.80	5	3.00	0.73	0.68	−0.08
Isla Vionta	13	12.40	2.25	5	2.71	0.68	0.86	−0.08

GenePop. Population genetic differentiation was quantified with F_{ST} that compares populations based upon allele frequencies and with R_{ST} that takes allele size under the stepwise mutation model into consideration (Slatkin, 1995).

Genetic parameters were tested for differences between island and mainland populations by exact tests in FSTAT (10 000 permutations). The relationship between genetic (A_r, H_o, H_s and F_{IS}) and topographic parameters (island size, distance to mainland) was explored with permutation tests for linear models with the R package lmPerm (Wheeler, 2010). The relationships between pairwise combinations of log-transformed genetic differentiation $[(F_{ST}/(1-F_{ST})), (R_{ST}/(1-R_{ST}))]$ and geographical distance among mainland and island populations were explored with the Mantel tests, with 10 000 permutations, using FSTAT. We also performed a power analysis using R package pwr (Champely, 2012).

Microsatellite genotype frequencies conformed to expectations under Hardy-Weinberg equilibrium at all loci in all populations, except locus Pb66 at Rua and locus Pb47 and Pb55 at Noro where significant deficiencies of heterozygotes were noted ($P < 0.01$). Genotypic linkage disequilibrium tests showed no significant values. Genetic parameters are summarized in table 1. The raw genotypic data are available as online supplement (Supplementary Information).

We found significant lower values of all genetic parameters in island than in mainland populations, except for F_{IS} (mean A_r: mainland 4.090, island 2.786, $P < 0.05$; mean H_o: mainland 0.813, island 0.590, $P < 0.05$; mean H_s: mainland 0.807, island 0.589, $P < 0.05$; mean F_{IS}: mainland −0.007, island −0.001, $P > 0.5$). This result is in agreement with information from polymorphic enzyme loci and mitochondrial DNA sequence data for the same study system (Arntzen and Sá-Sousa, 2007). We suggest that the observed low level of genetic variation in islands is caused by long-lasting population isolation.

No association was found between island size and genetic diversity of *P. guadarramae* (A_r: $F_{[1,4]} = 0.567$, $P > 0.2$; H_o: $F_{[1,4]} = 0.788$, $P > 0.2$; H_s: $F_{[1,4]} = 0.086$, $P > 0.5$; F_{IS}: $F_{[1,4]} = 0.068$, $P > 0.5$). Underlying our study is the assumption that island size is a proxy to lizard effective population size, but this relationship is at best weak. For example, the Island Rua is small (5 ha) but carries a large lizard population, probably as a result of the available habitat in the shape of man-made dry stone walls constructed during an episode of sheep farming (Arntzen and Sá-Sousa, 2007; Băncilă et al., 2010). We observed no association between island distance to mainland and genetic diversity (A_r: $F_{[1,4]} = 5.416$, $P > 0.05$; H_o: $F_{[1,4]} = 0.492$, $P > 0.2$; H_s: $F_{[1,4]} = 4.516$, $P > 0.1$; F_{IS}: $F_{[1,4]} = 0.234$, $P > 0.5$) nor an association between geographic distance and genetic differentiation ($r_{\text{m-Fst}} = 0.167$, $r_{\text{m-Rst}} = 0.269$), although at $P = 0.07$ the latter value is marginally significant. The most probable explanation for the lack of signal is low power (0.16) due to small sample sizes. To yield power of 0.8 would require an island sample size of 105.

Genetic diversity is a sensitive indicator of isolation suffered by natural populations and is successfully applied here to an explicit spatial-temporal setting. On the continent habitat destruction and fragmentation pose important threats on *Podarcis* lizards (Pascuala et al., 2011) and increasingly populations are restricted to small and isolated fragments that can be seen as 'habitat islands' (Prugh et al., 2008; Bennett and Saunders, 2010). Although our study is limited by small sample sizes and low power, the results are promising and could be interpolated to a smaller spatio-temporal scale and therewith serve as an early warning signal for *Podarcis* lizards in fragmented landscapes.

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