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Fluctuating asymmetry is a function of population isolation in island lizards

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Abstract

We measured the level of fluctuating asymmetry (FA) in head shape, head scalation and femoral pores in two lizard species (*Podarcis bocagei* and *Podarcis hispanica*) from 13 islands and 15 mainland localities in the Ria de Arosa archipelago of north-western Spain. Given the recent geological history of the region, the degree of isolation to which lizard populations have been subjected can be ordered along a spatio-temporal gradient, yielding the following hypotheses to be tested: FA will be higher (1) in island populations than in mainland populations; (2) on remote islands than on islands close to the mainland; (3) on small islands than on large islands. Molecular genetic data suggest that *P. hispanica* is autochthonous in the Ria de Arosa, whereas *P. bocagei* is a more recent arrival. Therefore, we predict also (4) a higher level of FA in *P. hispanica* than in *P. bocagei*. Statistically significant results were obtained for head-shape asymmetry, supporting the second and the fourth hypotheses. With an overall meristic asymmetry index, none of the hypotheses were corroborated, whereas for certain independent meristic traits, the first, the third and the fourth hypotheses were partially supported. Both head shape and meristic traits constitute precise measures of FA, but FA is more convincingly expressed in head shape and in single meristic traits than in overall meristic traits asymmetry. We conclude that FA reflects population isolation and may be a good indicator of developmental instability. It seems worthwhile to test for FA in a landlocked system under environmental and genetic stress, for the purpose of conservation biological assessments.

Introduction

The current rate of species loss throughout the world has drawn considerable attention from both conservation biologists and policy makers to the process of population extinction (May, Lawton & Stork, 1995). Theory predicts that species may be at a greater risk of extinction when the populations they are in are small and isolated, for reasons of (1) 'genetic stress', that is loss of population genetic variability through inbreeding and genetic drift; (2) 'stochasticity', that is the increased chance of population size going under the limit of sustainability; (3) limited or no 'rescue effect' that is, lack of immigration from nearby populations (Soulé, 1986; Allendorf & Luikart, 2007). Population isolation is, however, a relative term, governed not only by the parameters of time and space but also by the environment that would be encountered by individuals dispersing from one surviving population to the other. Island configurations may provide a particularly suitable setting for studying the process of population extinction because (1) islands often support small, isolated populations that are vulnerable to extinction (Eldridge *et al.*, 1999); (2) populations and distances between them are more readily defined than on the continent (MacArthur & Wilson, 1967; Gillespie & Roderick, 2002).

Podarcis bocagei and *Podarcis hispanica* are two ground-dwelling lizards, commonly distributed over the north-west of the Iberian Peninsula including the Ria de Arosa archipelago in Galicia, Spain. The Ria de Arosa is a so-called drowned river valley. The marine inundation process started *c.* 14 000 years ago and continues up to the present day (Pannekoek, 1966). Thus, islands far from the mainland and those with deep bathymetry show longer periods of isolation than islands near the coast and those with shallow bathymetry. As such, island populations of lizards experienced long and strong population isolation as opposed to mainland populations that remain interconnected in a network, with the strength of the connections determined by the habitat suitability matrix.

The identification of a simple and sensitive method for the reliable, efficient and cost-effective estimation of population isolation would be valuable, in particular for conservation biology. Fluctuating asymmetry (FA) refers to random variation from symmetry in bilateral organisms where symmetry of the character is the norm. It has been used as a measure of the ability of organisms to undergo normal development in spite of environmental or genetic stress (Møller & Swaddle, 1997). Here, we investigate whether FA can be used as such a tool in an island setting of more or less isolated lizard populations. The general aim was to test whether FA is related to island size and to different spatio-temporal degrees of population isolation. If such relationships hold in an island system, we would be interested to investigate whether FA could also be used to infer lizard population isolation in less tractable semi-continuous land-locked systems. Specifically, we tested the predictions that FA will be higher (1) in island populations than in those from the mainland; (2) on remote islands than on islands close to the mainland; (3) on small islands than on large islands.

A phylogeographic analysis using enzyme, microsatellite and mitochondrial genetic data suggests that the last glacial refugium of *P. bocagei* was in northern Portugal and that the current distribution in western Galicia represents a recent range expansion (Pinho, Harris & Ferrand, 2002; C. Pinho, pers. comm., 2005). Because *P. bocagei* is a recent invader of the area whereas *P. hispanica* is autochthonous for longer (Pinho, Ferrand & Harris, 2006; Arntzen & Sá-Sousa, 2007), a fourth hypothesis to be tested is that FA will be higher in *P. hispanica* than in *P. bocagei*.

Material and methods

The material used in this study consists of 288 preserved adult specimens of *P. bocagei* ($n = 85$) and *P. hispanica* ($n = 203$) from the Netherlands Centre for Biodiversity Naturalis (formerly National Museum of Natural History, Naturalis), Leiden, the Netherlands. The material was collected in July and August 1962–1964 by M. S. Hoogmoed and W. J. Roosdorp (see Brongersma & Pannekoek, 1966) and in July 2002 by M. S. Hoogmoed, J. M. Oliveira and the senior author from 13 islands in the Ria de Arosa and 15 mainland localities surrounding the Ria (Fig. 1). The surface area of the islands varies from 1.2 to 700 ha and the distance to the mainland ranged from 0.22 to 6.64 km (Table 1). No correlation was found between surface area of the islands and their distance to mainland either for *P. hispanica* ($r^2 = 0.08$, $P = 0.87$, $n = 6$) or for *P. bocagei* ($r^2 = 0.31$, $P = 0.49$, $n = 7$). The islands were classified as small versus large, meaning smaller or larger than average island size (10 ha), not considering the larger islands Isla Salvora (190 ha) and Isla de Arosa (700 ha) when calculating the average island size. The islands were also classified as close versus remote to the mainland, based on the average distance (3.43 km) between the closest (0.22 km) and most remote island (6.64 km).

For each lizard, digital photographs were taken in triplicate from both lateral and top sides of the head. Thirty-two 'landmarks' were positioned on the left side image and the mirror images of the right side using the software TpsDig 1.18 (Rohlf, 1999*a*; Fig. 2). We also counted the following head scales: supratemporal (TPL), supraocular (SOC), supraciliary (GRA), supralabial (SBL) and sublabial (SUB) (Arnold, Arribas & Carranza, 2007; Vervust *et al.*, 2008; Fig. 3) and the femoral pores (FP) along the ventral side of both left and right thighs (Cole, 1996). In order to quantify measurement error (ME), the landmark procedure was triplicated and the counting of meristic characters was duplicated.

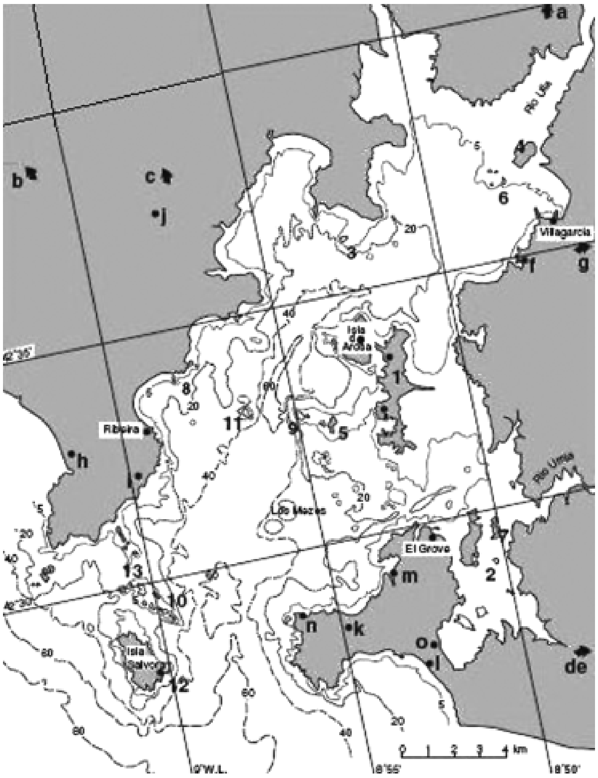

Figure 1 Situation map and bathymetry of the Ria de Arosa archipelago, Galicia, Spain after Cadée (1968). Island and mainland localities with lizards (*Podarcis bocagei* and *Podarcis hispanica*) studied are indicated by numbers (1–13) and letters (a–o), respectively. See Table 1 and Arntzen & Sá-Sousa (2007) for details.

For each individual, FA in head shape was measured as the Procrustes distance (Klingenberg & McIntyre, 1998). The landmark data for the head were transformed into shape variables using the least-square Procrustes superimposition algorithm in the CoordGen6f, part of the Integrated Morphometrics Package (IMP; Sheets, 2003). We used analysis of variance on Procrustes-aligned coordinates (Procrustes ANOVA) in order to analyse (1) the overall asymmetry (FA) of head shape; (2) directional asymmetry (DA; Van Dongen, Lens & Molenberghs, 1999) and (3) ME. Individuals and head side were entered as random and fixed effects, respectively. FA is measured by the side × individual interaction, DA is expressed by the main effect for sides and ME is expressed by the residual term. When DA was detected, shape data were corrected according to

Table 1 *Podarcis* populations under study, with sample size, map code, surface of the islands where they reside and distance to the mainland

				Sample size	
Locality	Map code	Island surface (ha)	Distance to mainland (km)	*Podarcis bocagei*	*Podarcis hispanica*
Isla de Arosa	1	700	3.28	12	0
Isla Beiro	2	4.84	0.37	6	0
Isla Benencia	3	1.77	0.95	10	0
Isla Cortegada	4	47.7	0.23	6	0
Isla Jidoiro Arenoso	5	7.96	5.77	19	0
Isla San Bartolomé	6	5.44	1.15	4	0
Isla Toja Pequeña	7	14.7	0.46	8	0
Isla Coroso	8	1.20	0.22	0	7
Isla Jidoiro Pedregoso	9	8.70	6.64	0	42
Isla Noro	10	3.18	4.44	0	22
Isla Rua	11	4.67	3.67	0	54
Isla Sálvora	12	190	3.80	0	6
Isla Vionta	13	12.4	2.25	0	7
Bridge over Ria Ulla, near Puentecesures	a	–	–	2	0
Playa de Louro, N. of Ria de Muros y Noia	b	–	–	1	0
Mirador de la Curotá near windmill plant of Serra de Barbanza	c	–	–	2	1
Between Mostero da la Armenteira and Mostero del Poio	d	–	–	6	7
Mostero de la Armenteira, NE of Sanxenxo	e	–	–	7	0
Punta Preguntoiro	f	–	–	2	0
Quarry near Caldas de Reyes	g	–	–	0	2
Between Vilar and Corrubedo	h	–	–	0	8
Castineiras, Mount Los Paramos	i	–	–	0	2
Mirador de la Curotá	j	–	–	0	11
Mirador, Con de la Siradella, Peninsula del Grove	k	–	–	0	1
Playa de Lanzada	l	–	–	0	2
Punta Moreiras, Peninsula del Grove	m	–	–	0	1
Punta San Vincente, Peninsula de Grove	n	–	–	0	9
Quarry in Coto de Caza San Martin near Ardia	o	–	–	0	21
Total				85	203

For exact localities and voucher material see appendix S1in Arntzen & Sá-Sousa (2007).

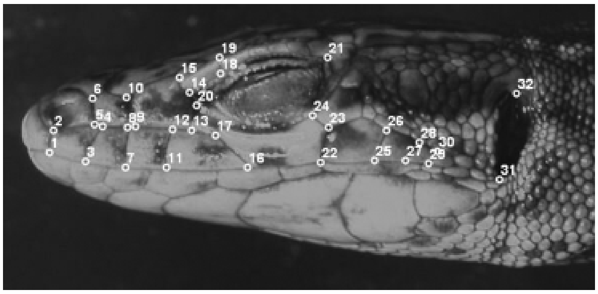

Figure 2 Definition of landmarks on the left side of the head of a lizard (genus *Podarcis*).

Klingenberg & McIntyre (1998). The degrees of freedom are that for ordinary ANOVA multiplied by shape dimensions, which is twice the number of the landmarks minus the four degrees of freedom that are lost during superimposition. To determine anti-asymmetry (AS; Van Dongen *et al.*, 1999), we visually examined scatter plots for each landmark of the left and right vectors after superimposition by the Procrustes algorithm. The clustering of vectors would suggest AS and is the equivalent to a binomial distribution of left/right differences. We also examined whether size dependence affected the asymmetry of shape with multivariate regression (Jobson, 1992).

For each meristic trait, FA was estimated as the absolute difference in counts between left and right sides (Palmer & Strobeck, 2003). The meristic traits asymmetries were analysed on a one-by-one basis and also combined per individual in the composite index CFA3 of Leung, Forbes & Houle (2000). The meristic traits were analysed for FA, DA and ME using a two-way mixed model ANOVA (Palmer & Strobeck, 1986; Palmer, 1994). The model design is similar with Procrustes ANOVA, except that the dependent variables are counts of meristic traits. AS was assessed through departure from a normal distribution, summarized by kurtosis and skewness estimates. When DA was detected, meristic data were corrected according to Van Valen (1962). The association between FA and size was tested using the linear regression of the signed asymmetry (R−L) on character size defined as $[(R_i+L_i)/2]$, for each meristic trait. Because the magnitude of skewness was small and

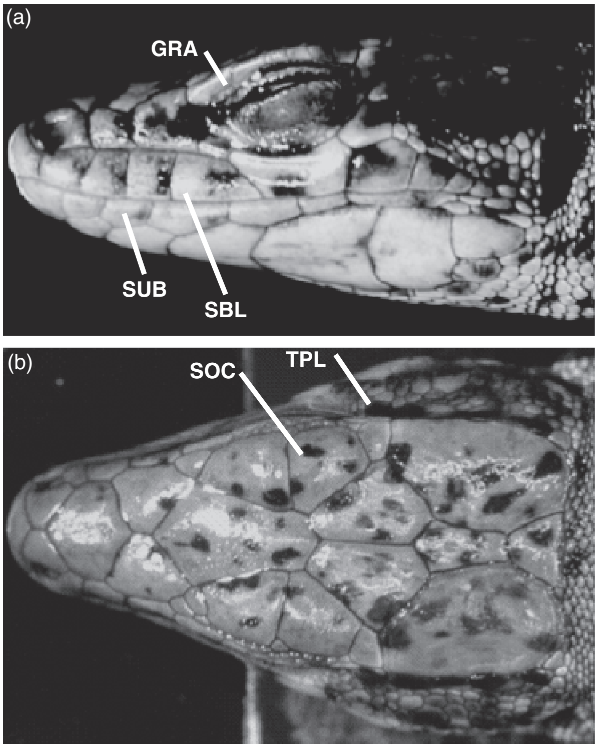

Figure 3 Side (a) and top (b) view of a lizards head showing the scales recorded in this study. TPL, supratemporal; SOC, supraocular; GRA, supraciliary; SBL, supralabial and SUB, sublabial.

statistical significance was attained as a result of large sample size for some characters, we did not exclude the characters concerned from the analysis. Moreover, we chose to not correct for size dependence because size could be correlated with the factor(s) under investigation and such a correction may thus mask an association with FA (Palmer & Strobeck, 1986).

As lizards were sampled from discrete populations, the dependent variables were potentially correlated, by virtue of genealogy. We, therefore, tested the effect of location (mainland vs. island), distance to mainland (close vs. remote islands), island size (small vs. large islands) and colonization history of the two lizard species (resident vs. recent colonizer) on head-shape asymmetry, independent meristic traits FA and composite index CFA3 of FA with linear mixed models (LMM; Cnaan, Laird & Slasor, 1997) using a weighted least-squares fitting procedure (Cho & Park, 2005). For each set of statistical tests, we applied a sequential Bonferroni adjustment in order to balance the type I/ type II error distribution (Holm, 1979).

Results

The Procrustes ANOVA of head-shape variation showed that FA and DA were statistically significant in both species (Table 2). Significant FA was detected for all meristic characters in both species, whereas significant DA was found for GRA and SUB in *P. bocagei* and for TPL and SUB in *P. hispanica*. For all meristic characters and also for head shape, the mean square of FA exceeded ME. Visual inspection of data plots revealed no evidence for clustering of vectors of shape asymmetry that would have suggested AS (results not shown). Significant levels of skewness for the distribution of right minus left differences were found for one meristic character (SOC) in *P. bocagei* and four meristic characters in *P. hispanica* (SOC, GRA, SBL and SUB; Table 3). All meristic characters showed a significantly leptokurtic distribution, except SUB in *P. bocagei* and FP in both species (kurtosis larger and smaller than unity, respectively). Because there was no platykurtosis or bimodality, we eliminate the possibility of AS acting as a confounding factor in our tests for FA. The regression of signed shape asymmetry on the mean centroid size was not significant for either *P. bocagei* ($r^2 = 0.045$, $P = 0.68$) or *P. hispanica* ($r^2 = 0.012$, $P = 0.86$). Significant size dependency of FA was found for SOC in *P. bocagei* and for GRA in *P. hispanica* (Table 3).

The analyses with LMM indicated that distance to mainland explained significant amounts of variation in head-shape asymmetry, FA being higher on remote islands than on islands close to the mainland for both species (data in Table 4 and test results in Table 5). No significant difference in head-shape asymmetry was found between lizard populations inhabiting small and large islands or between islands and mainland populations. Also, levels of FA did not vary significantly among island–mainland location, remote–close to mainland islands or small–large islands when the meristic traits where combined in a composite FA index. When meristic traits were analyzed on a one-by-one basis (Table 5), location showed a significant effect on FP asymmetry in *P. hispanica*, with asymmetry higher on islands than in mainland populations (mean difference ± SE: 0.104 ± 0.204). In *P. bocagei* island size had a significant effect on SUB asymmetry (0.279 ± 0.128) with a higher asymmetry in small islands compared with populations from large islands. Significant differences in head-shape FA were also found between resident lizards (*P. hispanica*) and recent colonizers (*P. bocagei*), with a higher level of FA in the former (0.085 ± 0.42, $F_{[1,36.245]} = 3.912$, $P = 0.049$). Colonization history also showed a significant effect on SOC (0.264 ± 0.122, $F_{[1,12.689]} = 4.664$, $P = 0.032$) and SBL (0.234 ± 0.081, $F_{[1,25.446]} = 7.871$, $P = 0.009$), FA being higher in resident species for both meristic traits.

Discussion

Conservation biologists need rapid, reliable and cost-effective techniques for detecting reductions in fitness inflicted by demographic and environmental insults. Human disturbance has caused an increase in stochastic fluctuations in the size of several animal and plant populations and has led to changes in their genetic structure. These factors could determine the loss of genetic variation and deepen inbreeding depression, causing fitness declines (Crnokrak & Roff, 1999; Frankham, 2005; Allendorf & Luikart, 2007). Recognizing the presence of genetic and environmental stresses before their effects become deleterious is an important but difficult task for conservation biology (Gilligan *et al.*, 2000; Lens *et al.*, 2000). To assist in this endeavor, we chose to assess developmental instability (Pertoldi *et al.*, 2006) in a natural, mostly undisturbed system that presents population isolation over a wide temporal–spatial axis.

Table 2 Procrustes ANOVA and two-way mixed model ANOVA for testing the significance of between-side* individual interaction [fluctuating asymmetry (FA)], between-side variation [directional asymmetry (DA)] and between-replicates variation [measurement error (ME)] in *Podarcis bocagei* (top panel) and *Podarcis hispanica* (lower panel) lizards from the Ria de Arosa archipelago, Galicia, Spain

	Fluctuating asymmetry			Directional asymmetry			
Trait	MS	d.f.	*F*	MS	d.f.	*F*	Between replicate variation
Podarcis bocagei							
Head shape	0.0023	1, 4960	52.946**	0.121	1, 60	54.030**	0.00004
Meristic characters							
TPL	1.224	1, 84	52.015**	1.694	1, 84	1.384^{NS}	0.024
SOC	3.561	1, 84	403.532**	0.662	1, 84	0.186^{NS}	0.009
GRA	0.867	1, 84	36.839**	4.648	1, 84	5.362**	0.024
SBL	0.187	1, 84	21.246**	0.003	1, 84	0.016^{NS}	0.009
SUB	0.497	1, 84	84.488**	0.497	1, 84	4.421**	0.006
FP	1.193	1, 84	19.323***	0.497	1, 84	0.416^{NS}	0.061
Podarcis hispanica							
Head shape	0.0007	1, 1176	1.367**	0.055	1, 60	77.4 13**	0.0005
Meristic characters							
TPL	0.849	1, 201	25.989**	3.298	1, 201	3.912*	0.033
SOC	1.254	1, 201	28.017**	1.424	1, 201	1.138^{NS}	0.045
GRA	1.299	1, 201	14.925**	1.612	1, 201	1.240^{NS}	0.087
SBL	0.085	1, 201	8.540**	0.005	1, 201	0.059^{NS}	0.010
SUB	0.624	1, 201	100.304**	3.494	1, 201	5.601**	0.006
FP	1.017	1, 201	15.298***	0.493	1, 201	0.484^{NS}	0.066

The denominator used to calculate F-values for the main side effect is the mean square of individual side interaction, and for the individual side interaction it is the mean square (MS) of measurement error. For meristic characters codes see text.

Statistical significance after sequential Bonferroni's correction.

$^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

NS, not significant; d.f., degrees of freedom.

Table 3 Means of the left-right differences ± standard error (SE) with skewness and kurtosis estimates and mean meristic character size values and slope of the regression of left–right differences on character size, for *Podarcis bocagei* (top panel) and *Podarcis hispanica* (lower panel) lizards from the Ria de Arosa archipelago, Galicia, Spain

	Signed difference (R−L)					[(R+L)/2]		
Character	Mean ± SE	Skewness	*P*	Kurtosis	*P*	Mean ± SE	Slope ± SE	*P*
Podarcis bocagei								
TPL	−0.157 ± 0.118	−0.946	NS	4.642	**	6.379 ± 0.250	−0.014 ± 0.054	NS
SOC	0.081 ± 0.204	2.992	**	18.777	**	5.426 ± 0.150	0.466 ± 0.142	***
GRA	0.264 ± 0.105	0.432	NS	0.705	**	6.178 ± 0.069	−0.236 ± 0.124	NS
SBL	0.011 ± 0.046	0.076	NS	2.901	**	5.113 ± 0.034	0.332 ± 0.168	NS
SUB	0.083 ± 0.075	−0.111	NS	0.443	NS	5.155 ± 0.089	−0.012 ± 0.098	NS
FP	−0.077 ± 0.120	0.143	NS	0.559	NS	16.247 ± 0.321	0.002 ± 0.040	NS
Podarcis hispanica								
TPL	0.057 ± 0.058	−0.364	NS	2.841	**	6.212 ± 0.174	0.023 ± 0.026	NS
SOC	−0.035 ± 0.082	2.741	**	21.224	**	4.631 ± 0.077	0.043 ± 0.076	NS
GRA	0.013 ± 0.064	−1.343	**	9.277	**	5.874 ± 0.062	−0.277 ± 0.084	**
SBL	0.016 ± 0.021	0.595	**	7.402	**	5.036 ± 0.045	0.049 ± 0.036	NS
SUB	0.119 ± 0.045	1.317	**	6.846	**	4.919 ± 0.058	−0.099 ± 0.065	NS
FP	−0.042 ± 0.072	0.135	NS	0.137	NS	15.513 ± 0.190	0.017 ± 0.026	NS

For meristic characters codes see text. Statistical significance after sequential Bonferroni correction

$P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

NS, not significant.

Table 4 Mean, standard deviation (SD), range and coefficient of variation (CV) of head shape asymmetry and the CFA3 composite index for fluctuating asymmetry for meristic traits in *Podarcis bocagei* and *Podarcis hispanica*, from the Ria de Arosa archipelago, Galicia, Spain

	Mean	SD	Range (min–max)	CV	Mean	SD	Range (min–max)	CV
Podarcis bocagei								
Location	Island ($n=65$)				Mainland ($n=20$)			
Head shape	0.252	0.015	0.098–0.660	0.465	0.225	0.017	0.115–0.418	0.340
CFA3	13.538	0.481	8–26	0.286	12.7	0.768	8–22	0.270
Distance to mainland	Remote ($n=31$)				Close ($n=34$)			
Head shape	0.290	0.025	0.098–0.660	0.469	0.220	0.015	0.109–0.507	0.403
CFA3	13.400	0.773	8–26	0.316	13.657	0.609	8–21	0.264
Island size	Large ($n=26$)				Small ($n=39$)			
Head shape	0.271	0.028	0.109–0.660	0.518	0.241	0.016	0.098–0.537	0.416
CFA3	12.360	0.770	8–20	0.311	14.275	0.594	8–26	0.263
Podarcis hispanica								
Location	Island ($n=138$)				Mainland ($n=65$)			
Head shape	0.348	0.026	0.089–1.678	0.877	0.270	0.033	0.098–2.087	0.990
CFA3	13.957	0.327	8–28	0.275	13.477	0.619	8–40	0.371
Distance to mainland	Remote ($n=131$)				Close ($n=7$)			
Head shape	0.517	0.078	0.218–0.753	0.399	0.339	0.027	0.089–1.678	0.908
CFA3	13.985	0.333	8–28	0.272	13.429	1.784	8–21	0.352
Island size	Large ($n=13$)				Small ($n=125$)			
Head shape	0.222	0.017	0.124–0.308	0.284	0.361	0.028	0.089–1.678	0.879
CFA3	13.308	0.916	8–20	0.248	14.024	0.349	8–28	0.278

One commonly used estimator of developmental instability is FA. Although several measures of FA have been proposed (Lens *et al.*, 2000), we estimated FA using a classical method by counting meristic traits and the more recent and complex method of 'geometric morphometrics' (Rohlf, 1999*b*). Because analyses that combine information across traits may be more reliable detectors of stress than those based on single traits (Leary & Allendorf, 1989; Watson & Thornhill, 1994), we used also an overall meristic asymmetry index. Meristic traits are fixed early in development and thus are not sensitive to age or body size variation (Taning, 1952; Leary *et al.*, 1985). Moreover, they can be measured with a small ME, thus yielding accurate measures. Geometric morphometrics, defined as the fusion of geometry and biology (Bookstein, 1982), aims to preserve the physical integrity of the shape; it avoids collapsing shape in series of linear or angular measures that would not include information about the geometric relations of the whole. From the various approaches used in geometric morphometrics, the landmark method was found to be a reliable, verifiable and, hence, widely accepted mean of recording and comparing shapes. Obviously though, landmark data provides no information on the surfaces that lie in between the landmarks (Richtsmeier, Deleon & Lele, 2002).

We tested the usefulness of FA as an indicator of population isolation in a particularly informative spatial–temporal setting, namely two species of lizards in the Ria de Arosa archipelago. Because the ME in our system was small, both head shape and meristic traits constituted reliable measures of FA. Statistically significant results were obtained for head-shape asymmetry, supporting the second and the fourth hypotheses. For the overall meristic asymmetry index, none of the hypotheses were corroborated, whereas for independent meristic traits, the first, the third and the fourth hypotheses were partially confirmed. FA may be trait specific because different traits will have different stress buffer capacities, varying susceptibilities to environmental stressors and differing ontogenetic patterns and timing of developmental asymmetry (Swaddle, 2003). Accordingly, FA determined over integrated units may be a more accurate reflection of the true asymmetry than single traits, because it is evaluated over more than one axis (Polak & Starmer, 2001).

Population isolation has been shown to increase inbreeding and genetic drift in for example reptiles and rodents (Leung, Dickman & Moore, 1993; Sarre, Dearn & Georges, 1994; Cunningham & Moritz, 1997; Madsen, Stille & Shine, 1997) and has been associated with an increase in FA (Soulé, 1967; Sarre & Dearn, 1991; Sarre, 1996). We observed higher FA in the character FP in insular lizard populations of Ria de Arosa archipelago than on the mainland, in line with hypothesis 1 and previous studies. The remoteness of the island, in our system reflecting the period of population isolation, led to a higher degree of head-shape asymmetry in both lizard species (hypothesis 2). Even though oversea dispersal has been inferred from population genetic data (one lizard from the island Jidoiro Pedregoso to the neighbouring Jidoiro Arenoso; Arntzen & Sá-Sousa, 2007), the insular populations by and large seem to be isolated from one another.

The third hypothesis predicts that FA is more pronounced on small islands than on large islands. Here, the

Table 5 Two-way linear mixed-model analysis testing the effect of location (mainland vs. island), distance to mainland (close vs. remote islands) and island size (small vs. large islands) on head shape asymmetry, asymmetry in each meristic traits and the CFA3 composite index of fluctuating asymmetry

	Podarcis bocagei			*Podarcis hispanica*		
Source	d.f.	*F*	*P*	d.f.	*F*	*P*
Location						
Head shape	1, 15.329	0.756	NS	1, 12.371	1.55	NS
Meristic character						
TPL	1, 14.764	0.981	NS	1, 20.246	0.052	NS
SOC	1, 20.387	1.844	NS	1, 20.097	1.397	NS
GRA	1, 8.463	1.300	NS	1, 12.595	1.401	NS
SBL	1, 8.873	0.123	NS	1, 7.897	0.159	NS
SUB	1, 14.754	0.417	NS	1, 9.716	0.204	NS
FP	1, 8.321	0.260	NS	1, 20.169	4.046	*
Overall Meristic FA						
CFA3	1, 15.331	0.065	NS	1, 16.162	0.563	NS
Distance to mainland						
Head shape	1, 18.633	6.092	**	1, 25.545	4.83	*
Meristic character						
TPL	1, 4.207	0.462	NS	1, 4.636	2.325	NS
SOC	1, 3.578	0.544	NS	1, 3.166	0.157	NS
GRA	1, 5.568	0.343	NS	1, 5.736	0.677	NS
SBL	1, 6.954	0.863	NS	1, 28.372	0.301	NS
SUB	1, 5.113	0.04	NS	1, 24.208	0.84	NS
FP	1, 6.267	0.014	NS	1, 13.653	0.073	NS
Overall meristic FA						
CFA3	1, 58.63	1.247	NS	1, 21.317	0.107	NS
Island size						
Head shape	1,5.643	1.001	NS	1, 15.136	1.238	NS
Meristic character						
TPL	1, 3.389	0.321	NS	1, 13.348	0.704	NS
SOC	1, 6.453	1.769	NS	1, 12.380	2.489	NS
GRA	1, 5.097	0.357	NS	1, 17.380	0.753	NS
SBL	1, 6.732	1.111	NS	1, 41.709	0.109	NS
SUB	1, 6.58	4.712	*	1, 35.070	0.416	NS
FP	1, 6.234	0.002	NS	1, 13.800	0.08	NS
Overall meristic FA						
CFA3	1, 10.829	1.461	NS	1, 18.706	0.331	NS

Populations were included as a random factor.

Statistical significance after sequential Bonferroni's correction.

$^{*}P<0.05$, and $^{**}P<0.01$

NS, not significant; d.f., degrees of freedom.

underlying assumption is that island size is a reliable indicator of (effective) population size (King, 1987). Small populations may endure more environmental stress (e.g. harsh competition due to limited resources and/or co-existing with competitor species) (MacArthur & Wilson, 1967 Crnobrnja-Isailović, Aleksić & Bejaković, 2005) and genetic stress (e.g. erosion of genetic variation affecting disease resistance) than large populations (Young, Boyle & Brown, 1996; Frankham, 1998; Crawford *et al.*, 2001). One factor limiting lizard population size on the smaller islands of the Ria de Arosa may be the absence of natural wells, where fresh water is only generated under conditions of fog. A particularly dense population of *P. hispanica* was found on Rua. This medium-sized island (*c.* 4.7 ha) has a lighthouse and has been farmed. The building and the dry stone walls bordering the pasture fields provide shelter to the lizards. The current high lizard density, compared with other islands where shelter opportunities are more limited, suggests that predation by birds of prey may constitute an important factor limiting lizard population size. Despite the outlier Rua, we observed a negative relationship between FA in sublabial scales and island size. A similar result was found by Sarre & Dearn (1991), also in lizards.

The most plausible hypothesis on the biogeographical history of *Podarcis* in the Ria de Arosa Archipelago (Pinho *et al.*, 2002; Arntzen & Sá-Sousa, 2007) is that *P. hispanica* was present on the proto-islands when the Ria was formed at the end of the last glacial period (14 000 years BP) and that *P. bocagei* colonized the eastern part of Ria during a cold period that lasted from the 15th to the 19th century (Pethick, 1984; Dias *et al.*, 2000). This 'Little Ice Age' involved a temporary decline in the sea level, with which islands over the current 10 m isobath became temporarily reconnected to the mainland. Hypothesis 4 predicted asymmetry in the resident species *P. hispanica* to be higher than that in the recent colonizer *P. bocagei*. The hypothesis was confirmed. Although we attribute the result to longer periods of isolation of insular *P. hispanica* than of *P. bocagei*, it can not be ruled out that the difference is species specific, for example derived from an underlying low genetic variation in *P. hispanica*, resulting from a population bottleneck during unfavourable conditions in the Pleistocene. To fully disentangle the several driving forces that would explain the observed data, a much larger study is required than that presented here. Moreover, the specific environments presented by the various islands may be responsible for some of the inferred developmental instability, through the disruption of co-adapted gene complexes (Clarke, 1993). A novel environment may exert a new and strongly directional selection pressure. The islands differ from one another not only in distance from the mainland or size but also vegetation cover. Vegetation varies from sparse on sandy islands (Jidoiro Arenosa and Vionta), to rocky islands with dense vegetation restricted to the base of granite rock boulders (Coroso, Jidoiro Pedregoso, Noro), to densely vegetated (Beiro, Benencia, San Bartolome), whereas the mainland localities and the larger islands such as Arosa and Salvora provide the lizards with more or less similar, mostly rocky habitats. Losos, Warheit & Schoener (1997) related morphological change in lizards to vegetation characteristics and Soulé (1967) speculated about a possible positive relationship between ecological complexity of a habitat and the degree to which the selective regime is stabilizing the development of an individual.

FA is a sensitive indicator of genetic and environmental stress suffered by natural populations (Leary & Allendorf, 1989; Parsons, 1990; Sarre *et al.*, 1994) and is successfully applied here to an explicit spatial–temporal setting. However, population isolation can also come about as a result of habitat fragmentation and such systems will generally be more difficult to assess (Anciães & Marini, 2000). We are keen to investigate whether FA could also be used to assess (lizard) population isolation in less tractable semi-continuous land-locked systems or 'habitat islands' such as nature reserves, or hills and hillocks surrounded by agricultural terrain.

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