

# Assessing factors involved in determining fluctuating asymmetry in four insular populations of the Balearic lizard *Podarcis lilfordi*

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**Abstract.** In animals, developmental stability is frequently assessed by the level of fluctuating asymmetry. Several environmental and genetic stress factors can increase the developmental instability in a population. Anyhow, the use of fluctuating asymmetry as a measurement of developmental instability and its relationship to other measurements of genetic and environmental stress, remain controversial. We studied this subject in the Balearic lizard, *Podarcis lilfordi*, and examined four populations inhabiting different coastal islets of Minorca (Balearic Islands, Spain). These populations show clear ecological and genetic differences that allowed us to study factors potentially determining the level of fluctuating asymmetry. We examined the following predictions: (1) fluctuating asymmetry will increase on smaller islands; (2) fluctuating asymmetry will increase on islands with a higher lizard density; (3) fluctuating asymmetry will increase on islands with more heavily parasitized lizards; and (4) populations with higher inbreeding levels would be more susceptible to environmental stress. Our results would partially support the fourth prediction of a higher fluctuating asymmetry in populations with lower genetic variability and, consequently, a higher inbreeding level. Probably, other environmental factors modulate this relationship.

Key words. developmental stability, island, genetic variability, environmental stress, parasites, *Podarcis lilfordi*.

## Introduction

Developmental stability is viewed as an individual's ability to buffer its development against random perturbations (VAN DONGEN & LENS 2000a). It is frequently assessed by the level of fluctuating asymmetry (FA), which corresponds to the small and random differences between the right and left sides of an otherwise bilaterally symmetrical character (VAN VALEN 1962). Several environmental and genetic types of stress can increase the degree of developmental instability. Fluctuating asymmetry today tends to be considered a population parameter, playing no role at individual level (PALMER 1999). However, it is also used as an indicator of an individual's quality (MØLLER & SWADDLE 1997, VAN DONGEN & LENS 2000b).

This study tries to approach the subject of FA using the Balearic lizard, *Podarcis lilfordi*, as a model species. We examined four populations of *P. lilfordi* inhabiting different coastal islets around the island of Minorca (Balearic Islands, Spain). Although ectotherms are generally considered to be good indicators of environmental stress (LEARY & ALLENDORF 1989), there are few studies in the developmental stability of reptiles. In addition, lizards have many metric (such as limb or head dimensions) and meristic (such as scales, femoral pores or subdigital lamellae) paired characteristics that are relatively easy to measure (SOULÉ 1967), and they are excellent models for ecological or eco-

morphological studies (PIANKA 1986, LOSOS 2009). At population level, the small islets around Minorca present ideal scenarios to study the effects of genetic and ecological factors that may influence development stability, because they host isolated lizard populations in relatively simple ecosystems. Also, developmental instability measured by FA is, presumably, more evident in small island populations because they may be exposed to more environmental (MAC ARTHUR & WILSON 1967) and genetic stress (YOUNG et al. 1996, FRANKHAM 1998, CRAWFORD et al. 2001).

In the absence of human intervention, gene flow between islands is limited or absent. CRNOBRNJA-ISAILOVIĆ et al. (2005) argued that isolated populations were more prone to be impacted by conditions such as bottleneck effect, long-term strong selection, low effective population size, or crowding effects, which may lead to higher inbreeding levels, periods of intense directional selection, and extreme homozygosity. In addition, MØLLER & SWADDLE (1997) argued that all these factors "have been shown to break down the balance of a genome and reduce the ability of the organism to buffer its developmental pathways against the production of random errors". Hence, theory suggests a positive correlation between FA and levels of inbreeding as it would increase homozygosity (MØLLER & SWADDLE 1997).

In addition, small populations may also withstand more environmental stress through, for example, competition for

limited resources and/or co-existence with predator species (MAC ARTHUR & WILSON 1967, CRNOBRNJA-ISAILOVIĆ et al. 2005). Due to increased genetic stress, small populations may be more susceptible to environmental stress. While not-inbred populations do not suffer from certain changes in the environment, inbred populations perceive them as stressful, which indicates a causal link between genetic and environmental stress (KRISTENSEN et al. 2006). Such connection can lead to synergetic effects between both types of stress, resulting in environmental conditions that would normally be benign becoming detrimental (KRISTENSEN et al. 2006). To assess the impact of competitor or predator species, we deliberately selected populations where we have four combinations of presence or absence of rats and gulls available for study. These two species are often perceived as significant agents in the alteration of many ecological communities, particularly on islands (PÉREZ-MELLADO 2002, ORO & MARTÍNEZ-ABRAÍN 2007). However, *Mus spretus* LATASTE, 1883 is present on Colom Island, and even though mice are not normally recognized as predators of Mediterranean lizards, they are known as predators of *Teira dugesii* (MILNE-EDWARDS, 1829) on Atlantic islands (P. WAGNER, pers. comm., 19 April 2014).

Among non-genetic factors, parasite load and population density have been the most widely studied factors in relation to FA. MØLLER & SWADDLE (1997) and MØLLER & MANNING (2003) presented large sets of evidence from a range of vertebrate taxa and concluded that population density could result in higher levels of FA, presumably generated by the reduced availability of resources at higher densities and increased competition. However, other authors did not find such a relationship between population density and FA (WIENER & RAGO 1987) or in general (KRUUK et al. 2003).

Another potential source of environmental stress is parasitism, which is considered to be detrimental to host individuals. It also has been linked with the occurrence of asymmetry in various traits (MØLLER 1996 and references therein). Nevertheless, little is as yet known about the possible causative nature of the relationship between parasite load and developmental instability (POLAK 1997). In two different reviews, MØLLER (1996, 2006) supported the hypothesis that there was a positive relationship between parasite load and FA. He also warned that the link between both variables were expressed in various ways, did not imply causation, and that the damage caused to the host was related to the parasite's virulence. As BROWN & BROWN (2002) proposed, developmental instability itself would affect the immune system and cause greater susceptibility to parasites. They also proposed that poor environmental conditions (e.g., poor nutrition, pollutants) would promote developmental instability, and it follows that these might also promote the parasite load of hosts by increasing their exposure to parasites, perhaps as a result of positive effects on local parasite abundance. BROWN & BROWN (2002) concluded that these possibilities have not been ruled out in most of the studies that have shown a relationship to exist between parasite load and asymmetry (MØLLER 1996,

MØLLER & SWADDLE 1997). In theory, populations that are exposed to more stressful conditions should exhibit higher levels of parasite load and FA. Notwithstanding, several studies did not find such a correlation (BERGSTROM & REIMCHEN 2005 and references therein).

In lizards, the isolation of insular populations has been shown to lead to increased inbreeding and genetic drift (MADSEN et al. 1996, MADSEN et al. 2000), and the size of an island has been associated with the degree of FA occurring there (SOULÉ 1967, SARRÉ & DEARN 1991, SARRÉ 1996, VERVUST et al. 2008, BĂNCILĂ et al. 2010). However, no such increase in FA was found in small island populations of *Podarcis muralis* (CRNOBRNJA-ISAILOVIĆ et al. 2005). At individual level, some studies related the asymmetry of hatchlings to temperatures during egg incubation (QUALLS & ANDREWS 1999, JI et al. 2002, ZHDANOVA & ZAKHAROV 2006), physiological performance (MARTÍN & LÓPEZ 2001, LÓPEZ & MARTÍN 2002, VERVUST 2008, but see also WARNER & SHINE 2006), or mating and reproductive success (MARTÍN & LÓPEZ 2000, 2006, but see LAILVAUX & IRSCHICK 2006).

The objective of our work is to study the influence of genetic and non-genetic (density, competitor species and parasitism) factors on the degree of FA in both individuals and populations. We predict that FA will increase: (1) on smaller islands; (2) on islands with a higher lizard density; and (3) on islands with more heavily parasitized lizards. We also expect that populations with higher inbreeding levels would be more susceptible to environmental stress, that is, the impact of competitor species should increase with inbreeding levels.

## Material and methods

### Species under study

The Balearic lizard, *Podarcis lilfordi* (GÜNTHER, 1874) (Squamata, Lacertidae), is a medium-sized lizard endemic to the coastal islands and islets of Mallorca, Minorca and the Cabrera archipelago (Balearic Islands). Lizards were collected by means of noosing. For each individual, we recorded its age as adult or juvenile and sex, SVL, tail length (if original), and body weight. Because of the low number of juveniles captured, only data from adult individuals were analysed. Body condition was evaluated from the residual of the regression of log-transformed body weight versus log-transformed SVL.

We quantified the prevalence and intensity of infection by two different parasites, mites and haemogregarines. Both kinds of parasites were previously studied (GARRIDO & PÉREZ-MELLADO 2013a, b). Mites of *P. lilfordi* were provisionally identified as belonging to the genus *Ophionyssus*, while blood parasites were regarded as representing the haemogregarines group (GARRIDO & PÉREZ-MELLADO 2013a, b). Ectoparasites were counted at the site of capture with a 5× eyeglass, inspecting the whole body surface of the lizards. To assess the extent of blood parasitism we obtained blood samples by making a slight longitudinal cut

Table 1. Genetic data and environmental factors of each population under study: NPS: total number of polymorphic sites; Pi: nucleotide diversity; k: average number of nucleotide differences; S: island area (in hectares); Rats: presence (+) or absence (-) of the ship rat, *Rattus rattus*; Seagulls: presence (+) or absence (-) of seagull breeding colonies; D: Biotic capacity index; B: niche breadth index of vegetation and substrate cover; lizard density (mean  $\pm$  SE lizards ha<sup>-1</sup>; Biome: lizard biomass (kg ha<sup>-1</sup>). Source: PÉREZ-MELLADO et al. (2008).

Population	NPS	Pi	k	S	Rats	Seagulls	D	B	density $\pm$ SE	Biome
Aire	1	0.000200	0.50	29.80	-	+	6.10	5.58	4098.60 $\pm$ 586.60	32.54
Colom	4	0.00095	2.07	51.14	+	+	7.62	9.38	1615.50 $\pm$ 294.93	11.02
Binicodrell	0	0.00	0.0	0.29	-	-	0.57	3.84	1180.50 $\pm$ 264.45	7.59
Tosqueta	0	0.00	0	0.29	+	-	0.57	4.91	1703.00 $\pm$ 501.41	11.29

in the dorsal proximal side of the tail with a sterile scalpel. The blood of drop emerging allowed us to obtain a blood smear in situ. Specimens were always released at the site of capture. Blood smears on microscopic slides were air-dried in the field. In the lab, these slides were fixed with absolute methanol for 10 minutes and then stained with modified Giemsa for 20 minutes. Samples were analysed using an optical microscope at 400 $\times$ . The only blood parasites identified were haemogregarines. The density of haemogregarines was estimated on the basis of a total of 2,000 counted cells per sample. Their prevalence was estimated as the percentage of infected individuals on each island. Within each year, individuals were made recognisable marking them with colour pens before their release. Lizards already examined in previous years could be recognized by the scar of the cut in the tail. Balearic lizards were studied on four different islands: Aire ( $n = 133$  females, 183 males), Colom ( $n = 89$  females, 121 males), Binicodrell ( $n = 19$  females, 31 males), and Tosqueta ( $n = 8$  females, 33 males). In all cases, individuals were sampled during the springs and summers of 2007, 2008, 2009 and 2010. The relatively simple ecosystems of these islands allowed us to identify the ecological variables that differed between populations. We deliberately selected these four populations, because the ecological and genetic differences between them were already known (PÉREZ-MELLADO et al. 2008, see Tab. 1), allowing us to study the factors potentially determining their levels of FA.

#### FA assessment

We measured one metric (hind limb length, HLL) and six meristic traits from the left and right sides: the number of femoral pores on the thigh (FEM), the number of subdigital lamellae under the fourth toe (LAM), the number of supraocular scales (SPO), the number of supralabial scales (SPL), the number of infralabial scales (INL), and the number of submaxillar scales (SMX). To verify whether the traits met the requirements for assessing FA (see below), we took three repeat measurements of them in a sample of 30 lizards. Hind limb length was measured from the insertion point of each leg into the trunk to the base of the claw of the fourth toe while keeping the leg fully extended. We

took the measurements with digital callipers to the nearest 0.01 mm. Meristic traits were counted with a 5 $\times$  eyeglass at the site of capture.

For metric and meristic traits in which the absolute differences of right minus left ( $|R - L|$ ) sides are large (4–5 units), PALMER (1994) and PALMER & STROBECK (1986) described a test to establish whether the between-sides variation was significantly larger than the measurement error (ME). The test is a two-way ANOVA (sides  $\times$  individuals) and should be conducted routinely as part of any study of FA (PALMER & STROBECK 1986). This procedure tests for the significance of all between-sides variation relative to measurement error, including antisymmetry (AS) and FA. One advantage of factorial ANOVA procedures is that the significance of directional asymmetry (DA) can be tested at the same time as that of FA relative to measurement error. For meristic traits, where the difference between sides is small (1–2 units) and where a low level of scoring error may exist, ANOVA techniques do not work properly, though. PALMER (1994 and pers. comm.) therefore recommended applying a likelihood ratio or G-test (SOKAL & ROHLF 1995). If the test produces a statistically significant result, it still needs to be confirmed that the mean differences in the character state between both sides ( $|(|R - L|/n)|$ ) is greater than the mean difference between all measurements ( $|M_x - M_y|/n$ ), where 'n' is the number of replicate counts. In these cases, DA must be estimated with a one-sample t-test of the mean (R - L), because departures from normality can mask DA (PALMER 1994).

Once tested for DA and ME, tests for the presence of AS should also be conducted in studies of FA. Typically, AS shows a platykurtic or bimodal frequency distribution in a given population, while DA presents skewness (MØLLER & SWADDLE 1997). To check for kurtosis and skewness, we applied the Anscombe-Glynn and D'Agostino tests, respectively (ZAR 2010). Finally, PALMER & STROBECK (1986) suggested looking for a possible dependence of FA to body size, correcting for this dependence if necessary. PALMER (1994) pointed out that body size might reflect some aspect of body condition. Thus, checking for trait size may partially control the extent of influence by body condition and yield asymmetry estimates that are condition-independent. Accordingly, we chose to do without a correction for size dependence, because body size could be correlat-

The placement of this reference suggests all data are taken from these authors!

Table 2. Results of G-tests and the protocol (see PALMER 1994) to assess FA relative to ME from meristic traits whose average difference between body sides is low. Last row: results for one-sample t-test, testing for departure from the expected mean of  $(R - L) = \text{zero}$ . This method allowed discarding the presence of DA in the different characters.

	FEM	LAM	SPO	SPL	INL	SMX
$\bar{x} \pm \text{SE}$ of $ R - L $	0.75 $\pm$ 0.04	0.83 $\pm$ 0.05	0.07 $\pm$ 0.01	0.12 $\pm$ 0.02	0.19 $\pm$ 0.02	0.03 $\pm$ 0.01
Palmer's G-test	$G_5 = 34.89$ $P < 0.001$	$G_9 = 6.54$ $P < 0.001$		$G_2 = 7.72$ $P < 0.05$	$G_1 = 4.95$ $P < 0.05$	
$\bar{x} M1 - M2 $	0.03	0.49		0.01	0	
$\bar{x} (R - L)/2 $	0.074	1		0.14	0.07	
FA > ME	yes	yes	yes	yes	yes	yes
Fisher's t-test	$t_{511} = -0.35$ $P = 0.73$	$t_{348} = -1.01$ $P = 0.31$	$t_{521} = -0.93$ $P = 0.35$	$t_{518} = -0.12$ $P = 0.91$	$t_{522} = 0.19$ $P = 0.83$	$t_{522} = 0.89$ $P = 0.37$

ed with factors under study and such a correction might mask their association with FA (PALMER & STROBECK 1986, BĂNCILĂ et al. 2010).

For our purpose, we used a composite FA index (CFA), as the analysis of composite indices is more powerful than analyses based on single traits (MØLLER 2006, see discussion in LEUNG et al. 2000). In addition, analyses that combine information across traits should prove to be more reliable detectors of stress (LEARY & ALLENDORF 1989, WATSON & THORNHILL 1994). Therefore, after all traits had been analysed for FA, they were combined per individual in the composite CFA2 following the recommendations by LEUNG et al. (2000). Briefly, the FA value of each trait was divided by its average  $|FA|$ , so that all traits contributed equally to the CFA, and then  $|FA|$  values were summed across traits for each individual so that each individual achieved a composite FA score.

#### Statistical analysis

First, we ran a GLM model with CFA2 as the dependent variable, and island and mite and haemogregarine loads as independent variables. To avoid spurious differences that are actually caused by sex-dependent differences, we introduced this factor to the model. CFA2 data were Box-Cox transformed to obtain homoscedasticity. Then, as we obtained differences between insular populations, we ran another GLM model, introducing environmental (rats, seagulls and density) as well as genetic factors of populations (NPS, Pi and k; Tab. 1). Thus, we looked for the factors leading to differences in FA between islands. We tested for co-linearity of the explanatory variables and discarded any variable with a variance inflation factor (VIF) of  $> 3$  (ZUUR et al. 2010). Genetic variables showed VIF values higher than this threshold, for which reason Pi and k were deleted and NPS was introduced to the model, producing a final set of four variables.

The statistical analyses were carried out using the software R environment (ver. 2.12.1, R Development Core Team, 2010). We started out with saturated models, con-

sidering effects of all independent variables as well as all interactions between them. To determine the minimum adequate model (CRAWLEY 2007), we used a backward stepwise regression on the basis of deletion tests (CRAWLEY 2007). Non-significant factors and interactions were removed from the models until we obtained a minimal adequate model, taking AIC (Akaike's Information Criterion) into account at each step. Post-hoc comparisons were made using the multcomp package (HOTHORN et al. 2009). Effect sizes reported here are partially eta-squared ( $\eta_p^2$ ) that can be interpreted similarly as  $R^2$  (COHEN 1988). Like  $R^2$ ,  $\eta_p^2$  is a dimensionless measure of effect size that is independent of the degrees of freedom used in the analyses (TABACHNICK & FIDELL 2000).

#### Results Assessing FA

All meristic traits show an average difference between sides of  $< 1$  (Tab. 2). We therefore applied Palmer's recommended G-test to see if ME was larger than FA (Tab. 2). The result of the G-test was significant in that the mean of  $|R - L|/2$  was greater than the mean of  $|M1 - M2|$  for all traits except SPO and SMX where no G-test could be applied as there was no variation in the repeated measurements (100% repeatability of measurements; Tab. 2). For these traits, we obviously concluded that variation between sides could not be due to ME. Moreover, the normality of all meristic traits was tested to be exclusive of DA (see above), and we found that distributions were normal for all meristic traits (Tab. 2). For HLL (metric trait), the two-way ANOVA recommended by Palmer showed that DA was not present and that the variation due to non-directional asymmetry was higher than the variation due to ME (Tab. 3).

A visual inspection of data plots revealed no evidence for a clustering of vectors of shape asymmetry that could have suggested the presence of AS. Nevertheless, significant levels of skewness for the distribution of right minus left-differences were found for SMX, but not for the remaining traits (Tab. 4). All traits showed a distribution that

Table 3. Two-way mixed model ANOVA: testing the significance of between-side individual interaction (non-DA), between-side variation (DA), and between-replicates variation (ME) in *Podarcis lilfordi*. The asterisk (\*) indicates significant factors; NS indicates no significant factors.

	Df	Sum Sq	Mean Sq	F	P
side	1	0.00	0.001	0.0085	0.9265
Individual	25	1090.96	43.638	274.8742	< 0.001*
Side:Individual	25	28.37	1.135	7.1472	< 0.001*
Residuals	104	16.51	0.159		

  

Non-DA (FA and AS)				DA			Between-replicate variation (MS <sub>m</sub> )
Trait	MS	df	F	MS	df	F	
HLL	1.135	25,104	7.14*	0.001	1,25	0.0008 <sup>NS</sup>	0.159

was significantly leptokurtic (Tab. 4). Since there were no platykurtosis or bimodality, we eliminate the possibility of AS acting as a confounding factor in our tests for FA.

To sum our results up, a significant degree of FA was detected in all characters examined in *P. lilfordi*, except for the skewness found in SMX, probably due to the test being limited to just two individuals that were asymmetrical for this trait and as a consequence of the small extent of variation in this trait. We decided to discard SMX in further analyses.

#### FA analyses

In the GLM model with parasite loads, sex and island, all interaction terms were insignificant (all  $P > 0.05$ ), so they were excluded from the model. Moreover, we did not detect differences in FA between males (mean  $\pm$  SE = 6.01  $\pm$  0.34;  $n = 249$ ) and females (mean  $\pm$  SE = 4.68  $\pm$  0.28;  $n = 368$ ;  $F_{1,610} = 1.90$ ,  $P = 0.17$ ), and the effect size was very small ( $\eta_p^2 = 0.004$ ). Also, no correlation was found for the haemogregarine load ( $F_{1,610} = 0.03$ ,  $P = 0.87$ ). Both were excluded from the final model.

Mite loads showed no significant correlation with CFA2 ( $F_{1,610} = 2.52$ ,  $P = 0.11$ ) either, but were retained in the minimum model. We found strong differences among islands ( $F_{3,610} = 19.34$ ,  $P < 0.001$ ), but the effect size was moderate ( $\eta_p^2 = 0.094$ ). Post hoc tests revealed that Tosqueta (mean  $\pm$  SE = 12.68  $\pm$  1.07, range = 23.89) showed statistically higher rates of asymmetry than Binicodrell (mean  $\pm$  SE = 8.15  $\pm$  1.47, range = 60.52;  $t = 4.05$ ,  $P < 0.001$ ), Colom (mean  $\pm$  SE = 5.91  $\pm$  0.38, range = 27.89;  $t = 6.63$ ,  $P < 0.001$ ), and Aire (mean  $\pm$  SE = 5.14  $\pm$  0.26, range = 25.10;  $t = 7.52$ ,  $P < 0.001$ ). Binicodrell individuals were found to be different from those living on Aire ( $t = 2.68$ ,  $P = 0.04$ ). Binicodrell did not show differences from Colom ( $t = -1.80$ ,  $P = 0.26$ ). Lizards from Colom were not more asymmetric than those from Aire ( $t = 1.31$ ,  $P = 0.54$ ).

The GLM model with environmental and genetic factors exhibited no significance for either the interactions or the presence or density of seagulls (all  $P > 0.05$ ). Only NPS ( $F_{1,610} = 46.27$ ,  $P < 0.001$ ) and the presence of rats ( $F_{1,610} = 10.94$ ,  $P = 0.001$ ) were related to CFA2. Thus, populations

Table 4. Skewness (D'Agostino test) and kurtosis (Anscombe-Glynn test) estimates for each measured trait. The asterisk (\*) indicates significant factors.

Trait	Skewness	P	Kurtosis	P
LMP	0.69	0.48	3.81	< 0.001*
FEM	0.1813	0.26	4.080	< 0.001*
LAM	-0.0022	0.99	4.099	< 0.001*
SPO	-2.31	0.02	11.54	< 0.001*
SPL	0.19	0.24	9.97	< 0.001*
INL	1.59	0.11	7.37	< 0.001*
SMX	2.44	< 0.001*	14.69	< 0.001*

living without the company of rats and with more genetic variability were less asymmetric.

## Discussion

We used CFA2 to test some hypotheses on FA. We did not find any differences between sexes in the levels of FA in contrast to some previous studies where males were more asymmetric than females (MØLLER & SWADDLE 1997). However, our result might be a consequence of the chosen sampling design. According to the theoretical assumptions of FA, we can conclude that both sexes are exposed to similar levels of stress.

FA levels were significantly different between all four island populations, with the exception of Aire versus Colom where similar degrees of asymmetry were found. Although we had expected to find higher FA levels on smaller islands due to increased levels of inbreeding, there was a lack of difference between Binicodrell and Colom, probably due to unequal sample sizes and a wide range of FA values on Binicodrell. Density has been pointed out as severely affecting developmental stability and as a consequence increasing FA (see, for example, MØLLER & SWADDLE 1997, MØLLER & MANNING 2003), and very few studies have questioned this relationship (e.g., KRUK et al. 2003). In our study, this relationship is not as obvious as the theory would suggest. GLM showed no influence of the density

on CFA2, but this could be due to our small sample size of just four island populations. Aire Island had a much higher population density, yet produced the lowest FA values along with Colom. Among the other islands, with similar population densities, FA differed substantially (Tab. 1).

Immune system and parasitism also could be linked to FA in poor environmental conditions (MØLLER & SWADLE 1997 and see Introduction). But in our case, neither mites nor haemogregarines seem to be good predictors of the degree of asymmetry. Parasitism not only depends on immune system efficiency, but also on other ecological and environmental factors (BIAGGINI et al. 2009). It even depends on the behaviour of individuals within a population (GARRIDO & PÉREZ-MELLADO 2013a, 2013b, and references therein). In this sense, the **Binicodrell individuals provide a good example of how the absence of ectoparasites may still produce high FA levels**. Also, negative results could be a consequence of our sample design. Here we studied two kinds of parasites with different degrees of virulence (see Material and methods) and just four populations with very different intensities of infection.

According to our results obtained from four insular populations, genetic stress would be a more reliable indicator of the degree of asymmetry. Theory predicts that small populations may suffer more genetic stress than large populations due to the erosion of genetic variation (YOUNG et al. 1996, FRANKHAM 1998, CRAWFORD et al. 2001). This loss of genetic variation and deeper inbreeding depression can cause declining fitness (CRNOKRAK & ROFF 1999, FRANKHAM 2005, ALLENDORF & LUIKART 2007). Genetic variability was significantly higher in larger populations (Tab. 1). Amongst the two less asymmetrical populations, Colom and Aire, no differences in CFA2 were found, but Colom showed a comparatively higher genetic variability (PÉREZ-MELLADO et al. 2008). Despite this exception, our results are consistent with other studies and prove the influence of genetic variability on FA levels. We propose that genetic variability affects stability when it has declined below a certain level as a result of continued inbreeding. Above that level, as it appears to be the case on Aire and Colom, there is no relationship between FA and homozygosity. In any case, more research must be done in this respect since our results are based on just four insular populations.

Besides the effect of genetic stress, insular populations must also withstand great environmental stress when they co-inhabit their islets with the mentioned competitor-predator species. The gull, *Larus michahellis* (NAUMANN, 1840), is present on Aire and Colom (Tab. 1), and the lizards from these two localities presented the lowest CFA2 values. Presumably, gulls are not responsible for differences in the development between islands as the GLM model suggests. Our results are in line with some recent papers that argued that their negative impact on the population trends of most species is not so straightforward (e.g., ORO & MARTÍNEZ-ABRAÍN 2007).

The ship rat, *Rattus rattus* (LINNAEUS, 1758), is an alien species that could alter endemic populations by predated upon them, but also because they compete for resources

(CASE & BOLGER 1991). Tosqueta and Binicodrell showed different levels of FA, and the most major ecological difference between them is the presence of *R. rattus* on the former islet (Tab. 1). Reptiles exhibit a high vulnerability to rodent presence (TOWNS et al. 2006). Our results obtained from the four studied populations support those of KRISTENSEN et al. (2006). Thus, Tosqueta individuals could respond more sensitively to the presence of rats than Colom ones do, probably because Colom lizards have a higher level of heterozygosity (PÉREZ-MELLADO et al. 2008).

In summary, although genetic variability is considered the best predictor of the degree of asymmetry in populations, other stressors may modulate this effect, and in our case it is perhaps the presence of rats. If parasites are indeed a cause of asymmetry, it is possible that the average intensity of parasite load in the populations investigated by us does not affect the development of individuals. In summary, the only conclusion is that FA and parasitism are not related at population level in *P. lilfordi* on the islets examined. PALMER (2002) regards FA as a poor indicator of the quality of individual development, as there may be random deviations from symmetry. According to WARD et al. (1998), perhaps the relationship between parasitism and FA is not as general as suggested by other authors (MØLLER 1996, POLAK 1997, MØLLER 2006). There has been a recent debate on the importance of publication biases in our understanding of ecological processes (MØLLER & JENNIONS 2001 and references therein). FANELLI (2012a, b) argued that papers with negative results were disappearing from most disciplines in all animal and plant sciences. This is even more serious when dealing with relatively recent issues such as FA, where statistical methodology is being updated continuously. WARD et al. (1998) warn “that any interpretation based solely on the proportion of studies reporting a positive association may be misleading” and cannot but agree more.

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