Contrasting Adult Body-Size in Sister Populations of the Balearic Lizard, *Podarcis lilfordi*
(Günther 1874) Suggests Anthropogenic Selective Pressures

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RRH: ROTGER ET AL.— BODY SIZE DIFFERENCES IN INSULAR LIZARDS

ABSTRACT: Recently isolated populations offer a good biological model to infer the evolutionary forces responsible for the current divergences across populations. We coupled genetic, morphometric, ecological, and demographic analyses from three island populations of the endemic Balearic wall lizard, *Podarcis lilfordi*, (Balearic archipelago, Spain) to infer the mechanisms underlying the observed differences in body size. For each population, we described plant community structure, derived a biotic capacity index, and used individual-based data on 1369 lizards captured and released during six years (2009–2015) to estimate population density and body growth patterns. We used genetic data collected on 80 individuals (~27 for each population) to infer genetic divergences across islets and population history. Body size divergences cannot be explained by the ecological or population characteristics. Individual growth was slower in the smallest island, where lizards reached the largest average body size. In addition to having the highest density, results suggested that resource availability does not constrain asymptotic body size, but the speed at which individuals reach it does. The Approximate Bayesian Computation used to infer population history from genetic data supported the occurrence of two bottlenecks in the islet with the highest anthropogenic footprint. We emphasize the need to integrate ecological and genetic data and the importance of considering the effects of past human disturbance as an additional force in being able to model present island fauna.

Key words: Bottleneck; Human settlement; Individual-based data; Insular lizards; Microevolution; Size-dependence

DIFFERENCES in body size among animal populations can result from site-specific selective forces (Mayr et al. 1963) and random genetic processes such as genetic drift and founder effect (Kimura 1968). Selective forces can also lead to trait divergences among populations without necessarily changing the genetic background due to phenotypic plasticity

(Reznick et al. 2004; Clegg et al. 2008; Schoener 2011). In ectotherms, realized body size differences across populations are often ascribed to a plastic response to different nonexclusive causes. The main one is the climatic conditions (Aragón and Fitze 2014), but the differences observed in body size can also arise from differences in per capita resources (Wikelski and Trillmich 1997; McNab 2010), predatory pressure (Bassar et al. 2013), or from the direct or indirect effect of human activities (Grayson 2001).

Continental islands are good biological models to separate the relative contribution of different evolutionary forces, including those imposed by anthropogenic activities (Bochaton et al. 2017). Their geological formation and the different historical events are usually well documented by fossil records and historical relates. In addition, populations were formed from vicariance rather than colonization or founding events (Pretus et al. 2004; Terrasa et al. 2009), giving them distinct evolutionary patterns in comparison to the oceanic islands (Bessa-Silva et al. 2016). These features allow the integration of historical (e.g., fossils that document the presence of past predators and signs of recent as well as past anthropogenic activities) and current environmental selective pressures that shape the present-day phenotypic differences among neighboring populations. Given the complexity of past and present selective processes to assess how body size differences across island populations have been generated and are maintained, it is important to account for current genetic diversity, population history, ecological data, and population-specific life-history traits. The Mediterranean basin offers a good number of continental islands, many of them inhabited by endemic species of lizards of genus *Podarcis*, whose colonization and speciation history are in many cases well studied (Oliverio et al. 2000). Here, we considered differences in body size within a single species of insular *Podarcis*, the endemic Balearic wall lizard, *Podarcis lilfordi* (Günther 1874). The species is currently found in more than 40 islets surrounding Mallorca, Menorca, and Cabrera archipelago. They originated at

the end of the Messinian Salinity Crisis (5.33 Myr) after the flooding of the Mediterranean Sea that kept them isolated from continental areas (Brown et al. 2008; Terrasa et al. 2009). Although populations never connected again with continental counterparts, insular *P. lilfordi* populations were inter-connected repeatedly with each other during the major ice ages (Riss, 200,000 years ago and the Würm, 25,000 years ago), when sea level decreased 130 and 110 m respectively (Gracia and Vicens 1998). After minor events during which sea level decreased only enough to connect neighboring islets (Terrasa et al. 2009), all current populations finally diverged when Mallorca and the surrounding islets were definitely separate between 5000–8000 yr (Petitpierre et al. 1987). The populations off the coasts of Mallorca can be classified into two main genetic clades by their mitochondrial DNA: the genetic clade belonging to the western islets of Mallorca and the remaining populations from Mallorca (Terrassa et al. 2009; Pérez-Cembranos et al. 2020).

We focused on three neighboring populations sharing the same environment and belonging to the same genetic clade but with marked differences in body size of 8–10%. Our study aims to reveal the evolutionary forces responsible for the current divergences found across three isolated neighboring populations of the Balearic wall lizard. We gathered different data types to cover genetics and ecological and demographic aspects that allow us to infer the evolutionary forces potentially responsible of the observed size difference among these three populations.

We used neutral genetic markers (microsatellites) to describe the fine genetic structure of the three sister populations. If the difference in body size resulted from random genetic processes, e.g., founder effect or/and genetic drift, we expected to find differences in the genetic structure, and in particular in the level of heterozygosity (Piry et al. 1999). In contrast, a similar genetic composition would suggest an adaptive change or a plastic response in body size resulting from

islets-specific selective pressures (i.e., adaptive genetic diversity or phenotypic plasticity). Both density-dependence phenomena and environmental selective pressures generate clear predictions on which population should host the smallest lizards. We expected an inverse relationship between lizard density and body size and a positive relationship between trophic resources and body size. Moreover, if per capita resources that influence body size and all islets have limited resources, the more densely populated islet and/or less food resources should host the smallest lizards (Wikelski and Trillmich 1997). On the other hand, if the past and current human activity induced an adaptive shift in body sizes, we expect an inverse relationship between human pressure and body size, with the smallest lizards found in the islet with more anthropogenic pressure (Pregill 1986). The hypotheses outlined above are not mutually exclusive, e.g., selection and random processes might act together, but comparison will help to build the most likely scenario that has generated and is maintaining the observed body size differences.

MATERIAL AND METHODS

Study Area, Study Species, and Ecological Characteristics

We considered three islets off the southern coast of Mallorca less than four km apart (Fig. 1): Moltona, Na Guardia, and the small islet of Es Curt. The three islets have scarce and similar predatory pressure, although the presence of rats have been observed erratically only in Na Guardia (personal observation), occasional bird predation has been also documented (Cooper et al. 2009). *P. lilfordi* is the unique lizard species in those islets. Islets differ in size, the larger islet is Moltona measuring five hectares, followed by Na Guardia (two hectares) and Es Curt (0.3 hectares). Male *P. lilfordi* are ca. 10% larger than females, but body size and size sexual dimorphism can vary across populations (Salvador 1980, 1986; Pérez-Cembranos et al. 2020). Newborn lizards measure about 30 mm long and laboratory data suggest that females begin to breed between the first and second year at a snout–vent length (SVL) of ca. 50 mm (Castilla and

Bauwens 2000). None of the islets are currently inhabited by humans, but the medium-size islet, hosting the smallest sized lizards, is the closest to urban area and harbor. It has experienced a continuous anthropogenic disturbance since a small human settlement established during the Punic era (4th century BC) that lasted about 300–400 yr (Guerrero 2000). Similar to many small islets around Mallorca, the three islets have evidence of a more recent human presence linked to some stonemasonry activities dated between 17th and 19th century (Salvà-Matas 2013). The degree of disturbance of this activity is not clear and it presumably changes according to the islet considered (Gornals et al. 2015). The present-day disturbance is related to the tourism industry and the three islets are regularly visited during the summer months. The interaction between lizards and tourists is restricted to the small sandy beaches of the islets.

To characterize the ecological context and provide a proxy of resource availability in each island, we studied the vegetation assembly of each islet following the syntaxonomical classification to determine the vegetation communities (Rivas-Martínez et al. 2001). We counted the number of plant species present in each island and measured the proportion of plants species with fleshy fruits. This proportion is important because lizards feed on the flowers and fruits produced by these species and pollinators are attracted by flowers. Finally, we calculated the index of biotic capacity, D (Cheylan 1992), which is used as an approximation of the biotic potential of the island: $D_i = \log (S_i \times a_i)$, where S_i and a_i are the surface and maximal altitude of the i -islet (Parlanti et al. 1988; Pérez-Mellado et al. 2008).

Demographic Traits and Population Structure

Sampling and density estimates.—Data for individual size were collected at Na Guardia and Es Curt from 2010–2015, while data collection began a year earlier in Moltona. In each islet, lizards were captured in pitfall traps during three-day capture-recapture sessions in October and April. Individual lizards were caught in geo-referred pitfall traps positioned along paths and

vegetation edges (see Ruiz de Infante et al. 2014). Each captured lizard was weighed, measured from snout to vent with a ruler to the nearest 0.5 mm, photographed using a digital camera for individual identification (Moya et al. 2015), and subsequently released at the same location of capture. Individuals were sexed by the inspection of femoral pores and by counting the number of row ventral scales in lizards with SVL < 50 mm (Lecomte et al. 1992; Rotger et al. 2016). Differences in body size were measured for adult individuals only (SVL $\geq$ 50 mm; Castilla and Bauwens, 2000). In order to avoid pseudoreplication in this analysis, we considered the body size measure at first capture. We calculated islet and sex specific mean values of SVL. Two samples *t*-tests were performed for males and females pairing the populations (Moltona–Na Guardia, Moltona–Es Curt, and Na Guardia–Es Curt). Sexual size dimorphism was calculated using the asymptotic size of each sex estimated by the growth curves (see below). The sexual size dimorphism index was measured as [(asymptotic size of the largest sex/asymptotic size of the smallest sex) - 1] (modified from Lovich and Gibbons 1992; Stephens and Wiens 2009). Population density was estimated in each October because October is a post-reproductive period where hatchlings are already observable, resulting in a density estimation that takes into account all age classes. Total lizard density (i.e., including all sizes) by island was estimated in October (2009–2015) during the post reproductive period using Spatially Explicit Capture–Recapture models (Efford 2004). These models use capture–recapture data collected with an array of detectors (traps) to estimate the lizard population density (Ruiz de Infante et al. 2014; Rotger et al. 2016).

Growth patterns.—The analysis of somatic growth was restricted to 1369 lizards of known sex and captured at least twice (355 males and 243 females from Moltona; 273 males and 100 females from Na Guardia; 209 males and 189 females from Es Curt). We tested three different characteristic models to describe growth in vertebrates: the von Bertalanffy’s growth

model (Fabens 1965; Schoener and Schoener 1978), the logistic-by-length model (Schoener and Schoener 1978) and the Schnute's equation (Schnute 1981). Models were implemented using a nonlinear mixed effects model (nlme package; Pinheiro et al. 2008) in the R statistical package (R Core Development Team 2017). We used Akaike's Information Criterion to select the most parsimonious of the three growth models (Burnham and Anderson 2004). Following Pinheiro and Bates (2000), we performed diagnostic tests to assess heteroscedasticity of residuals and over parameterization of random effects in the retained model. Once the appropriate growth model was selected, we considered sex and populations as fixed factors and estimated the characteristic growth constant (K) and the asymptotic size (L_∞ or y_2). Note that in doing so, the individual identity was treated as a random effect to account for individual variability using the nlme package (Pinheiro et al. 2008).

Survival analysis and female fecundity.—We estimated the annual survival of males and females of each islet through multisite capture–recapture models (Schwarz et al. 1993) using data from the October sessions. Time-dependent individual covariates, such as weight or body size, cannot be easily accommodated because covariate values are unknown when an individual is not caught (but see Bonner and Schwarz 2009). We followed the procedure in Fernández-Chacón et al. (2015) to estimate size-dependent annual survival in four state-classes identified by combining known aspects of *P. lilfordi* ecology with the length-by-age growth curve estimate for each population using all data available (see above “growth patterns”). The four states considered were: nonbreeding individuals or juveniles, individuals in their first reproductive season or subadults, adults which would be all the individuals between the size of second reproduction until the maximum asymptotic size estimated by the growth curve, and senior lizards which were individuals that have reached or exceeded their maximum asymptotic size. The probability to move from a class of size r to a class s , ϕ^{rs} , is comparable with the growth

rate. To account for the continuous growth, we constrained state-dependent survival estimates to be a linear function of the observed average value of each class (Fernández-Chacón et al. 2015). We use this approach to investigate the effect of sex and size on survival and recapture probabilities in each islet. Size effect was modeled as a linear trend, quadratic, and without size effect. The goodness of fit test of a general model was assessed with program U_CARE (Choquet et al. 2009) while model selection was carried out in program MARK (White and Burnham 1999). Models were compared using the corrected Akaike Information Criterion (Anderson and Burnham 2002). To accommodate model selection uncertainty, we calculated parameter estimates and their associated unconditional standard errors by model averaging (Burnham and Anderson 2002). In 2015, we measured female fecundity by moving 38 female lizards into the laboratory in the reproductive season from April to August (average 12 per islets; see details in Rotger et al. 2020). We considered the total number of eggs laid per female as a proxy of individual fecundity.

Genetic Diversity Across Populations

We used 15 specific microsatellite loci for a finer measure of population differences (Bloor et al. 2011). Genomic DNA was extracted from the tail tissue of 80 individuals (Moltona = 27, Na Guardia = 25 and Es Curt = 28) using standard phenol–chloroform protocols. PCRs were run in a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA), as in Bloor et al. (2011). Fluorescently labelled PCR products were run on an ABI 3130 DNA Sequencer (Applied Biosystems) with GeneScan-500 (LIZ) internal size standard. Fragment length was assigned using GeneMapper software v3.2 (Applied Biosystems) and visually rechecked. We used GENALEX V6.2 (Peakall and Smouse 2006) to calculate allele frequencies from standard measures of microsatellite diversity. For each islet, we measured genetic diversity, observed and expected heterozygosity, inbreeding coefficient, and assessed

deviations from the Hardy-Weinberg equilibrium. The procedure FreeNa was used to test the presence of null alleles for each locus using the ENA correction method (Chapuis and Estoup 2007). Population subdivision was evaluated using Bayesian assignment tests in STRUCTURE v2.3 (Pritchard et al. 2000) and by using STRUCTURE HARVESTER (Earl 2012) to determine the most likely value of the optimal number of distinct genetic clusters (Evanno et al. 2005). For the analysis we used ten independent runs assuming an ad-mixture model and correlated allele frequencies between 1–5 genetic clusters at 10^6 Markov Chain Monte Carlo repetitions and a burn-in period of 10^5 iterations. We complemented this first analysis with a Principal Components Analysis of the 15 microsatellite loci using *Adegenet* package (Jombart 2008) in R 3.1.0 (R Development Core Team, 2008). The degree of differentiation among genetic clusters was determined through an analysis of molecular variance, pairwise comparison of the Fixation Index-Statistic (F_{ST} ; Weir and Cockerham 1984), and the Fixation Index-Statistic based on microsatellite allele frequencies (R_{ST} ; Slatkin 1995) in GENALEX V6.2 (Peakall and Smouse 2006) to estimate the population differentiation. The presence of a possible gene flow among islands during the past one to three generations was assessed using BayesAss3.0 (Wilson and Rannala 2003). The algorithm was run for 50×10^6 iterations with a burn-in period of 10^6 iterations and a sampling frequency of 2×10^3 iterations. Our final run used delta values of migration rate = 0.15, allele frequencies = 0.45, and inbreeding coefficient = 0.35; to yield an average number of changes in the accepted range between 40–60% of the total chain length (Wilson and Rannala 2003).

Bottleneck Analyses and Populations' History

Program BOTTLENECK ver. 1.2 (Cornuet and Luikart 1996) was used to evaluate heterozygous excess over those expected in a population in equilibrium and the mode-shift in allele frequency (Luikart and Cornuet 1998). Coalescent simulations assumed a constant effective

population size to estimate heterozygosity under mutation-drift equilibrium. One-tailed Wilcoxon
 signed tests were performed to determine whether our data showed a significant number of loci
 with heterozygosity excess. Assumptions about the models of mutation for microsatellite loci are
 known to influence the significance of these tests (Peery et al. 2012); consequently, we used the
 infinite allele model and two-phase model to test heterozygosity excess. The two-phase model
 requires specifying the proportion of multi-step mutations, p_g , and the variance, which have been
 shown to influence Type I and type II error rates (Piry et al. 1999; Williamson-Natesan 2005).
 Analyses were run following Lukoschek (2018) using eight combinations of values: four values
 for the proportion of multi-step mutations (5%, 10%, 20% and 40%) and two values of variance,
 9.2 (Williamson-Natesan 2005) and 12 (Piry et al. 1999). We used the M-ratio approach to assess
 the presence of past (old) bottlenecks. The M value is expected to decrease in bottlenecked
 populations through stochastic loss of alleles, whereas the range of allele sizes remains relatively
 unchanged (Garza and Williamson 2001). We ran M_P_VAL setting the mean size of no single-
 step mutation and the proportion of multistep events to 3.1 and 0.22, respectively, following
 Peery et al. (2012). We simulated an equilibrium distribution of M value in a population of
 constant size assuming parameter values for the mean size, proportion of multi-step mutations,
 and θ , where $\theta = 4N_e\mu$ (N_e = prebottleneck effective population size; μ = mutation rate).
 Following Hurtado et al. (2012) we considered three values of multi-step mutations (0.05, 0.1,
 and 0.2; see Lukoschek 2018) and explored six values of θ ranging from 0.05 and 4, assuming 5
 $\times 10^{-4}$ mutations/generation/locus (Estoup and Angers 1998). M value, known as M critical was
 obtained after 10,000 simulations of a population at equilibrium using the software
 CRITICAL_M (Garza and Williamson 2001). A reduction in prebottleneck effective population
 size is suggested when the M-ratio falls below the simulated Critical value of M.

Finally, we used Approximate Bayesian Computation analysis (Cornuet et al. 2008) to infer population history (e.g., Chan et al. 2006; Lander et al. 2011) by testing different demographic scenarios (Appendix I) using the program DIYABC v2.1 (Cornuet et al. 2014). As suggested by the genetic structure (see Results), we used microsatellite markers to simulate four demographic scenarios characterized by the first separation of the Es Curt population (called in the analyses *Time 2*) while Moltona and Na Guardia diverged later (*Time 1*). At the present time (*Time 0*), the main difference between scenario 1 and 2 was the possible reduction of effective population size due the divergence of Es Curt. In scenario 3, we considered a possible bottleneck caused by the historical settlement, and in the last scenario 4 the combined effect of the historical human settlement with the other more recent bottlenecks (see Appendix I). Prior values of effective population for current populations were set as 10–5000 individuals with a uniform distribution, and an ancestral population was set as 10–10,000. Time of divergence was measured in generation time and prior values for time points were chosen according to the genetic results obtained (see results) and based on previous information about definitive isolation of the populations and historical events occurred on the islands (Ayuso 1981; Petitpierre et al. 1987; Terrasa et al. 2009; Appendix I). A million simulations were performed for each scenario. After a total of four million simulations, the most-likely scenario was evaluated by comparing posterior probabilities using logistic regression (Cornuet et al. 2008). We used a default generalized stepwise mutation model with a mean mutation rate of 1×10^5 to 1×10^3 (Ellegren 2000). Goodness of fit was assessed for each scenario by model checking using the Principal Component Analysis approach implemented in DIYABC. Finally, we assessed confidence in scenario choice by evaluating Type I and Type II error rates (Cornuet et al. 2008). One hundred test data sets were simulated using each of the four scenarios. The posterior probabilities of each scenario were evaluated for each of the simulated data sets. Type I and II error rates were the

proportion of times that a given scenario did not have the highest posterior probability among the competing once when assumed to be the best scenario (Type II error) or it did when assumed to be the wrong one (Type II error).

RESULTS

Ecological Characteristics

We listed a total of 124 species of vascular plants (see Supplementary Table S1). As expected, the number of species was positively associated with island surface, with 108 species at Moltona, 76 at Na Guardia and 15 at Es Curt. We recognized a zoning of four major vegetation types following the association type described by Rivas-Martínez et al. (2001): a coastal and nitro-halophytic community composed mainly by *Limonium spp.*, *Arthrocnemum macrostachyum* (Moricand) C. Koch, and *Suaeda vera* (Willdenow) Moquin-Tandon; a dune community composed mainly by *Lotus cytisoides* Linnaeus, and *Helichrysum stoechas* (Linnaeus) Moench; a shrub community made in majority by *Pistacia lentiscus* Linnaeus, and *Phillyrea media* Linnaeus; and an herbaceous community with a ruderal character composed mainly by *Lavatera arborea* Linnaeus, *Chenopodium murale* Linnaeus, and *Fumaria bastardii* Boreau (details in Supplementary Table S1). Moltona and Na Guardia had a similar plant composition sharing the same four vegetation types. The smallest islet, Es Curt, had only the halophytic community (Fig. 1). The biotic capacity index and the proportion of annual flowering plants decreased with islet size, being higher in Moltona and lowest in Es Curt (Table 1).

Demographic Traits and Population Structure

Moltona and Es Curt populations exhibit a similar mean SVL, 63.2 ± 5.7 mm (males: 64.8 ± 5.7 mm; females: 60.8 ± 4.3 mm) and 62.8 ± 5.7 mm (males: 67.0 ± 5.8 mm; females: 61.3 ± 3.9 mm), respectively. In Na Guardia, lizards were smaller compared to the other islets with a mean SVL of 60.2 ± 4.7 mm (males: 61.4 ± 4.7 mm; females: 57.1 ± 3.2 mm; Fig. 1). Body size

between males of each island were different (t -test_(Moltona-Na Guardia): $t_{340,259} = 7.994$, $P < 0.0001$; t -test_(Moltona-Es Curt): $t_{340,201} = 4.316$, $P < 0.0001$; t -test_(Na Guardia-Es Curt): $t_{250,201} = 11.185$, $P < 0.0001$). Females from Moltona and Es Curt were the only ones that we did not find a difference in body size (t -test_(Moltona-Na Guardia): $t_{226,93} = 8.332$, $P < 0.0001$; t -test_(Moltona-Es Curt): $t_{226,185} = 1.273$, $P = 0.204$; t -test_(Na Guardia-Es Curt): $t_{93,185} = 9.510$, $P < 0.0001$). In all islets, males were larger than females. Although sexual size dimorphism was very similar across populations, Na Guardia presented the largest sexual size dimorphism index (Moltona = 0.10; Na Guardia = 0.12; Es Curt = 0.11). Average lizard density measured in autumn was different among populations, with 680.31 $\pm$ 34.22, 1307.77 $\pm$ 84.97 and 1742.60 $\pm$ 71.14 individuals per hectare for Moltona, Na Guardia, and Es Curt, respectively.

Growth patterns.— Schnute equation was the best model describing the population growth patterns (see Supplementary Table S2). As expected, males had a larger asymptotic size and a greater characteristic growth constant ($P < 0.001$) than females regardless of the islet (i.e., no interaction between islet and sex; Fig. 3; see Supplementary Table S3 for model selection). Both asymptotic size and characteristic growth rate were different among males and females in all three populations. The average estimated asymptotic size, y_2 , was different across islets (Moltona: $y_{2\text{male}} = 74.9 \pm 0.2$ mm, $y_{2\text{female}} = 67.8 \pm 0.2$ mm; Na Guardia: $y_{2\text{male}} = 68.5 \pm 0.5$ mm, $y_{2\text{female}} = 61.4 \pm 0.5$ mm; Es Curt: $y_{2\text{male}} = 73.0 \pm 0.4$ mm, $y_{2\text{female}} = 65.9 \pm 0.4$ mm). The characteristic growth parameter, K , was smallest in Es Curt, but was similar between the other two islets. This indicates that individuals in Es Curt have a slower growth than in the other islands (Moltona: $K_{\text{male}} = 0.0023 \pm 0.00007$, $K_{\text{female}} = 0.0020 \pm 0.00008$; Na Guardia: $K_{\text{male}} = 0.0022 \pm 0.00022$, $K_{\text{female}} = 0.0019 \pm 0.00022$; Es Curt: $K_{\text{male}} = 0.00172 \pm 0.00015$, $K_{\text{female}} = 0.00136 \pm 0.00015$).

Survival probability and female fecundity.—

Survival probability was estimated using a total of 1680 individuals (Moltona: 400 males and 316 females; Na Guardia: 362 males and 187 females; and Es Curt: 173 males and 242 females) between 2009–2015 during the October sessions only. The goodness of fit indicated that the general model fit the data adequately in all populations (see Supplementary Table S4). Model selection procedure suggested an effect of sex and size on survival. As expected, large males had a slightly higher mortality than females, whereas female survival was constant independently of the size, except in Na Guardia. (Fig. 3 and Supplementary Table S5). However, survival probability for adult males was lower than adult females in Moltona and Es Curt. (Adults: $\text{Moltona}_{\text{male}} = 0.48 \pm 0.03$, $\text{Moltona}_{\text{female}} = 0.61 \pm 0.04$; $\text{Na Guardia}_{\text{male}} = 0.60 \pm 0.04$, $\text{Na Guardia}_{\text{female}} = 0.51 \pm 0.10$; $\text{Es Curt}_{\text{male}} = 0.77 \pm 0.02$, $\text{Es Curt}_{\text{female}} = 0.78 \pm 0.03$). Overall survival in relation to SVL was similar with the exception of the higher mortality of the largest (old) females in Na Guardia (0.32 ± 0.10).

The number of eggs laid by each female was measured in laboratory conditions on 38 females (Moltona: $n = 15$, Na Guardia: $n = 12$, Es Curt: $n = 12$; see details in Rotger et al. 2020). A total of 105 eggs were laid (Moltona: $n = 49$, Na Guardia: $n = 27$, Es Curt: $n = 29$) with an average number of 3.26 ± 1.75 eggs per female. No difference between the three islets was found (Likelihood ratio test: $\chi^2_2 = 2.99$, $P = 0.22$). Female body size was positively associated with the number of eggs in Moltona and Es Curt ($\beta = 0.172$, $z = 2.99$, $P = 0.003$ and $\beta = 0.140$, $z = 1.952$, $P = 0.05$) but not in Na Guardia ($\beta = 0.04$, $z = 0.607$, $P = 0.54$; see also Rotger et al. 2020). The smallest laying females measured 61 mm in Moltona, 51 mm in Na Guardia, and 62 mm in Es Curt, representing the (90), (83) and (94) percent of the average female asymptotic size in the respective islet.

We found between 8–19 alleles ($\bar{X} = 12.13$ alleles per locus). Estimates using the ENA correction method pointed to the absence of null alleles, scoring errors, or linkage disequilibrium (see details in Supplementary Table S6). The genetic diversity was high in all three populations and associated with island size. The level of inbreeding was generally small, although higher in the smallest islet Es Curt (relatedness = 0.083; Table 2). Pairwise comparisons showed genetic differentiation between Es Curt and the other two islands ($P < 0.001$). Fixation indices values indicated that Moltona and Na Guardia were closely related, while Es Curt was different (Table 3). The analysis of molecular variance within populations showed that 6% of variation was among populations and 94% within them ($P = 0.001$). Bayesian clustering suggested two clusters as the most likely number of genetic groups, with Moltona and Na Guardia assigned to the same cluster. Accordingly, the first component of principal component analysis (F1) on allelic frequencies differentiated Es Curt from the other two islets, while the second (F2) separated Moltona from Na Guardia (Fig. 4). BayesAss analyses indicated a high proportion of genotypes consistent with their own population [approximately (94), (97), and (98) percent in Moltona, Na Guardia, and Es Curt, respectively]. There was no recent gene flow among populations except between Moltona and Na Guardia, with one individual at Moltona estimated to have originated in Na Guardia ($m = 0.047$; 95% CI: 0–0.103).

Bottleneck Analyses and Population History

One-tailed tests of heterozygous excess were significant for the three populations under the infinite allele model (Table 4). Results for the two-phase model did not vary across the 4 x 2 combinations of multi-step mutations and variance of multistep mutations. The results were not significant in Moltona but they were in Es Curt and Na Guardia for the eight combinations (Table 4). There was no significant departure of mode-shift in allele frequency at any population. Simulations showed a strong decreasing trend in critical values of M for increasing proportion of

multi-step mutations. Increasing values of θ also decreased M critical values but the effect was weak (Table 5). Inference for M -ratio smaller than M critical differed across populations and combinations of proportion of multi-step mutations and θ (Table 5). No bottlenecks were inferred in Moltona, whereas five out of the 18 tests were significant in Na Guardia and ten were significant in Es Curt, where M -ratio was lowest. The number of significant M -ratio tests followed the pattern of heterozygous excess.

Approximate Bayesian Computation results indicated that the scenario 4 was the most likely scenario (Fig. 5). This assumed one ancient bottleneck and decrease in population size followed by a recent bottleneck in Na Guardia and another bottleneck in Es Curt. The highest posterior probability was found for scenario 4 (Fig. 5; 0.4635, 95% CI: 0.4466–0.4803) and the lowest for Scenario 3 (0.1015). The best scenario suggested that Na Guardia suffered at least two events of reduction of population size. The median values of effective population sizes for scenario 4 were 767 (95% CI: 343–988), 4720 (95% CI: 3900–4990), and 2950 (95% CI: 989–4890) for Es Curt, Moltona, and Na Guardia, respectively. The median values of the divergence times, *Time 1* and *Time 2*, were 610 (95% CI: 508–696) and 1600 (95% CI: 1050–1980) generations respectively (Supplementary Table S7). Considering a generation time of four to five years, divergence times were between 2440 and 3050 years ago for *Time 1* and between 6400 and 8000 years ago for *Time 2*. The estimated duration of the predicted ancient bottleneck at Na Guardia had a median of 93 generations (95% CI: 5–194), which correspond to between 372 and 465 years. In addition, this scenario suggests other more recent reductions in population size in Na Guardia and Es Curt: 327 (95% CI: 42–636) and 319 past generations (95% CI: 57–640), respectively. The duration of both bottlenecks was also similar: 27.4 (95% CI: 2–49) and 24.9 (95% CI: 2–49) generations in Na Guardia and Es Curt, respectively. Overall, scenario 4 had a high goodness of fit to observed data. Type I error for this scenario was relatively high (0.49)

compared with other scenarios whereas Type II error for was low (0.20–0.24) (Supplementary Table S8).

DISCUSSION

Lizard species are generally characterized by a remarkable degree of morphological differentiation among their populations (Losos et al. 1998; Jordan et al. 2005; Herrel et al. 2008) and island populations of the Balearic wall lizards is not an exception (Salvador 1980, 1986; Pérez-Mellado and Salvador 1988; Pérez-Mellado et al. 2008; Pérez-Cembranos et al. 2020). All differences may be typically caused by non-exclusive selective pressures such as predation, resource availability, or habitat type, among other factors. Here, we have collected ecological data, capture–recapture information, and used genetic techniques to try to disentangle the multiple potential factors that may be responsible for the morphological differentiation in adult body size in the endemic Lilford’s lizard. We selected three closely spaced, isolated, and genetically similar populations (same genetic clade; Terrassa et al. 2008, Pérez-Cembranos 2020). We have described the ecological, morphological, and fine-scale genetic differences found among them to assess the factors responsible for the divergences observed (Appendix II).

As expected by the island theory, Lilford’s lizards feed on several items including invertebrates, pollen, fruits, and plant parts (Pérez-Cembranos et al. 2016; Santamaria et al. 2019). Vegetation assemblages and the number of plant species were positively correlated with the surface area (Rita and Bibiloni 2003). The description of number of flowering species or plant species that produce fleshy fruits gives an idea of availability of resources for each islet both for the pollinators attracted by the flowers and the fruits available to be consumed by the lizards. Although the largest islet Moltona had the greatest number of plant species, both Moltona and Na Guardia showed the same vegetation structure. The smallest islet, Es Curt, showed the poorest floristic composition and the simplest vegetation structure. However, the food limitation in Es

Curt appears to constrain growth rate but not the expected asymptotic body size (see also Dunham 1978; Wikelski et al. 1997). Males and females from Es Curt and Moltona had similar realized body size. Surprisingly, Es Curt, the smallest islet with the poorest floristic composition, had the highest population density and showed the highest survival probability compared to the other populations. The combination of high densities and low resource availability could increase intra-specific competition, especially among males, promoting larger sizes and a higher degree of sexual size dimorphism (Buckley and Jetz 2007; Meiri 2007; Donihue et al. 2016). Reaching large size with low per-capita resources can only be possible at the expense of growth rate, which was the smallest among the populations (Andrews 1976; Le Galliard et al. 2005). The similar growth rate in Moltona and Na Guardia and the comparable diet composition of these two populations (Santamaría et al. 2019) confirmed that growth speed depends on the availability/type of food resources.

In ectotherm vertebrates, trait differences can be due to thermoregulatory constraints (Sagonas 2013; Aragón and Fitze 2014; Ortega et al. 2014). However, average climate is similar in all study islets and lizards in Es Curt and Moltona, which showed the larger differences in habitat type, reached similar adult size (see also Ortega and Martín-Vallejo 2019). Adult lizards in Na Guardia were on average more than 7 mm smaller than their relatives (Salvador 1980, 1986; Pérez-Mellado and Salvador 1988) and the estimated asymptotic sizes are far from those in the other two islets. One possible explanation is that extrinsic mortality is different and individuals in Na Guardia do not survive long enough to attain larger body size. However, results from the analysis of individual encounter histories bring little support for this hypothesis. Adult mortality was only 3% higher in Na Guardia than in Moltona, despite a drop in survival in old females in Na Guardia. The marked senescence in Na Guardia cannot be due to extrinsic mortality factors, such as predation or severe climate, which we would expect to affect males

with equal intensity. A possible explanation may be a cost of early reproduction. Despite females producing, on average, a similar number of eggs in the three islets, fecundity was increasing with female body size in Moltona and Es Curt, where the smallest laying females measured $\geq 90\%$ of the estimated asymptotic body size (Rotger et al. 2020). This relationship is typical of reptiles (Shine and Schwarzkopf 1992) and might be shaped by reproductive costs (Schwarzkopf 1994). Such a relationship, however, was absent in Na Guardia where small females produced a similar number of eggs than large ones and the smallest reproducing female was 83% of the estimated asymptotic body size. An early start of the reproduction and the absence of a size-dependent fecundity suggested a similar strategy to what observed in mainland populations or in populations under predatory/hunting pressure (Castilla and Bauwens 2000; Perera and Pérez-Mellado 2002; Hutchings 2004; Proaktor et al. 2007). A selective force acting against larger sizes may have selected for early breeding individuals changing the strategy of the individuals and, consequently, their realized body size.

The analysis of gene flow supported the isolated nature of the populations, with the exception of one migration event between the two closest populations, probably due to a recent translocation by tourists visiting the islets. In agreement with the detection of two clusters (Fig. 4), microsatellites revealed Es Curt and the combination of Moltona and Na Guardia as two genetically different populations. This was also confirmed by the fact that the alleles found in the Na Guardia population were part of the genetic pool of Moltona. Given that the three populations recently diverged from one common population, we suggest that this difference is probably due to a founder effect in the smallest islets because this population has private alleles different from Moltona. On the other hand, the neighboring populations in Moltona and Na Guardia showed to be genetically identical despite their morphological and vital rates differences. Possibly the vicinity of these two islets have promoted gene flow in the past, before the rise of sea level.

Despite Na Guardia showing a substantial degree of overlap with Moltona, its genetic diversity and the number of private alleles was lower probably as a consequence of a smaller island size or due to bottleneck events (Frankham 1996).

The analysis of possible historical scenarios supported a first change in population size, especially in Es Curt, following a first isolation event between Es Curt and Moltona + Na Guardia. The posterior isolation between Moltona and Na Guardia may explain why these two populations are genetically similar. The most supported scenario also suggested an important reduction in the effective population size in Na Guardia when the population diverged from Moltona ($Nb3$; Fig. 5). In addition, The Approximate Bayesian Computation analysis supported a bottleneck in Na Guardia that led to the loss of private alleles in this population and an important reduction in the effective population size dating back about 360 generations and lasted about 97 generations. This event matches the period and duration of the human settlement that occurred in Na Guardia during the Punic era (about 400 BC; Guerrero 2000). Remains of ceramic and iron objects in Na Guardia testified its use as a trading post for about 300–400 yr. Animal bones found during excavations indicate the presence of goats on the island during the Punic settlement (Guerrero 1997) and changes in the original flora could have occurred due to the grazing activity (see also Pafilis et al. 2013). The best scenario recorded two more recent events during which population size was drastically reduced in both, Na Guardia and Es Curt, for a short period of time. Despite the confidence intervals of the estimated periods at which bottlenecks occurred partially overlapped, the date and the duration of these recent bottlenecks could be associated with when the stone quarry activity between the 17th and the 19th centuries (Salvà-Matas 2013). Despite genetic data that support these scenarios, we cannot consider these events as a specific selective force against larger size. This is because the genetic information comes from neutral markers that, by definition, are sheltered from selective forces. Despite the effect of possible past

selective forces is unknown, it is undisputed that Na Guardia is the islet with the largest anthropogenic footprints compared to the other two populations. The short distance to the harbor, the presence of a small lighthouse, the high number of ruderal plants, the irregular presence of rats, and the remains of a past long-lasting human settlement (Guerrero 2000) suggest a greater anthropogenic disturbance compared to the other populations. A link between human presence/disturbance and the loss of phenotypic plasticity in body size is, however, a speculation and obviously we cannot directly test whether past and recent anthropogenic pressures selected for small body size in Na Guardia.

Body size in lizards is considered a highly plastic trait that responds rapidly to changes in selective pressures or food constraints (Herrel et al. 2008; Donihue et al. 2018). It is unlikely that this character remains constant long after the end of the selective pressures on large sizes. Past anthropogenic disturbance and historical human activities have been shown to cause permanent morphological changes in insular reptile populations (see Pregill 1986; Bochaton et al. 2017), but we do not have direct evidence to support this hypothesis. In conclusion, small lizards in Na Guardia showed a similar growth rate than the large lizards in Moltona and identical (neutral) genetic structure, a similar diet (Santamaria et al. 2019), but attain a different adult asymptotic size. The scenarios supported by the genetic information confirmed that something happened in Na Guardia that led to several reduction of the effective population size during many generations. Linking these reductions to the effect of human disturbance, both historical and recent (see Pregill 1986; Bochaton 2015), seems plausible but at this stage it is a speculation. Proximate and ultimate causes of size differences in this islet remain unknown. A Single Nucleotide Polymorphism analysis (Roesti et al. 2012) could help to assess the presence of selective forces on nonneutral parts of the genome. Our results stress the importance of considering

anthropogenic disturbance as an additional evolutionary force that may have shaped the present island fauna (Corti et al. 1999).

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APPENDIX I

Population History (Approximate Bayesian Computation Analysis)

In all scenarios Es Curt population diverged first from the ancestral population and Moltona and Na Guardia separation occurred later by proximity (Fig. 3). The four selected scenarios for comparison using Approximate Bayesian Computation Analysis were:

Scenario 1 (hierarchical split null model): since alleles detected in the Es Curt were more divergent from all the others, we assumed in these scenarios and the other four that the divergence of this population occurred before that the divergence of Moltona and Na Guardia. Es Curt merged at *Time 2*, with Na Guardia merging with Moltona more recently at *Time 1*. No reductions of size occurred in the scenario 1.

Scenario 2 (hierarchical split model with size reduction of effective population): this scenario is similar to scenario 1 but assuming that the divergence of Es Curt, and the ancestral population made up of Moltona and Na Guardia together, caused immediately a reduction of effective population. Dashed line in figure 3 shows the intermediate effective populations size after the separation.

Scenario 3 (Punic settlement): based on the genetic relationships among Moltona and Na Guardia, and also historical data, this scenario was considered the effect of the human settlement in Na Guardia islet during 200–400 years at 400 BC, coinciding with the Punic settlement arrived to the islet and with the duration of the settlement.

Scenario 4 (Punic settlement and recent bottlenecks): this scenario is similar to scenario 3 but, also took into account in the smallest islets (Es Curt and Na Guardia) the events that could have caused recent bottlenecks in these two populations following the results of the table 4.

Prior Distributions in Approximate Bayesian Computation Analysis to set the Demographic

Scenarios

Parameter	Minimum	Maximum
Effective population size		
Es Curt	10	5000
Moltona	10	5000
Na Guardia	10	5000
Ancestral population	10	10000
Time scale in generations		
<i>Time 1</i>	500	700
<i>Time 2</i>	1000	2000
Duration of bottleneck		
Bottleneck of Punic settlement	1	200
Recent bottleneck Na Guardia	1	50
Recent bottleneck Es Curt	1	50

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APPENDIX II

Results from the Genetic, Morphological and Ecological Data

	Moltona	Na Guardia	Es Curt
Genetic cluster	A	A	B
Evidence of bottleneck	No	Yes	Yes
Private alleles	2.6	0.87	1.27
Average population density	776	1546	1849
Number of plant species	108	76	15
Number of vegetation communities	4	4	1
Growth rate ♀	2.0×10^{-3}	1.9×10^{-3}	1.4×10^{-3}
Growth rate ♂	2.3×10^{-3}	2.2×10^{-3}	1.7×10^{-3}
Estimated asymptotic body size ♀	67.75	61.35	65.92
Estimated asymptotic body size ♂	74.86	68.46	73.02
Average number of eggs per female	3.26	2.25	2.41
Size-dependent fecundity	Yes	No	Yes

826 TABLE 1.—Physical and biotic characteristics of the three populations.

	Moltona	Na Guardia	Es Curt
Area (ha)	5.09	1.98	0.29
Maximum elevation (m)	11	9	5
Distance to the main island (m)	350	300	30
Distance to the nearest islet (m)	900	900	3500
Historical human settlement	No	Yes	No
Seabird nests (since year 2006)	Yes	Yes	Occasional
Biotic capacity index (D)*	4.03	2.45	0.37
Number of plant species	108	76	15
Ruderal plants	32	22	4
Meadow flowering plants	28	15	0
Species of fleshy fruit	12	8	3

827 *from Pérez-Mellado et al. 2008

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831 TABLE 2.—Genetic diversity within islets. In the table, we provide means $\pm$ 1 standard error.

Population	Sample size	Number of alleles	Efective number of alleles	Observed heterozygosity	Expected heterozygosity	Private alleles	Relatedn
Moltona	27	9.73 $\pm$ 0.64	5.62 $\pm$ 0.40	0.82 $\pm$ 0.03	0.81 $\pm$ 0.02	2.60 $\pm$ 0.47	0.015
Na Guardia	25	8.39 $\pm$ 0.57	4.57 $\pm$ 0.26	0.79 $\pm$ 0.04	0.77 $\pm$ 0.02	0.87 $\pm$ 0.19	0.039
Es Curt	28	5.87 $\pm$ 0.64	3.45 $\pm$ 0.39	0.62 $\pm$ 0.07	0.63 $\pm$ 0.06	1.27 $\pm$ 0.30	0.083

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834 TABLE 3.—Pairwise comparisons of Fixation Index-Statistic and Fixation Index-Statistic based
835 on microsatellite allele frequencies among the three lizard populations. *P* values are in brackets.

	Es Curt–Moltona	Es Curt–Na Guardia	Moltona–Na Guardia
Fixation Index	0.135 (0.001)	0.144 (0.001)	0.03 (0.001)
Fixation Index based on microsatellite allele frequencies	0.025 (0.037)	0.024 (0.034)	0.044 (0.005)

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TABLE 4.—Heterozygous excess in Moltona, Na Guardia and Es Curt populations. *P* values are shown for two models of mutation for microsatellites. In bold *P* values < 0.05.

Population	Heterozygous excess	Two-phase model								Infinite allele model
		Proportion of multistep mutations								
		0.05	0.10	0.20	0.40	0.05	0.10	0.20	0.40	
		Variance in the mean size of the multistep mutation								
		9.2	9.2	9.2	9.2	12	12	12	12	
Moltona	0.82	0.09	0.11	0.14	0.38	0.07	0.07	0.08	0.21	0.00
Na Guardia	0.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Es Curt	0.64	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.00

851 TABLE 5.—M-ratios and critical values for M for the proportion of multistep mutations and θ , where $\theta = 4N_e\mu$. $\theta = 0.01$ corresponds to
852 a pre-bottleneck N_e of 5, while $\theta = 4$ corresponds to a pre-bottleneck N_e of 2000, assuming $\mu = 5 \times 10^{-4}$. Bold values indicate assumed
853 bottlenecks i.e., where the M-ratio value is less than the critical value of M. n = sample sizes.

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			critical values for M																	
			Proportion of multistep mutations																	
			0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2	0.05	0.1	0.2
	<i>n</i>	M-ratio	$\theta = 0.05$			$\theta = 0.25$			$\theta = 0.5$			$\theta = 1$			$\theta = 2$			$\theta = 4$		
Moltona	27	1	0.92	0.87	0.81	0.90	0.86	0.79	0.88	0.84	0.77	0.86	0.81	0.74	0.84	0.78	0.71	0.81	0.75	0.69
Na Guardia	25	0.87	0.92	0.88	0.82	0.91	0.87	0.81	0.89	0.86	0.79	0.88	0.83	0.77	0.85	0.81	0.74	0.83	0.78	0.72
Es Curt	28	0.82	0.92	0.89	0.82	0.91	0.87	0.81	0.90	0.86	0.80	0.88	0.83	0.77	0.86	0.82	0.75	0.83	0.79	0.73

FIGURE CAPTIONS

FIG. 1.—Location of the three study islets on the southern coast of Mallorca (right top panel). Left top panel: south-western Mediterranean Sea, marker indicating the study location area. Bottom panels: zoom of each study islet.

FIG. 2.—Distribution of the Snout–Vent Length (mm) in males (on the top) and females (on the bottom) of the lizard study populations

FIG. 3.—Growth and survival differences among the three study populations. Somatic growth curve of males (black line) and females (grey line) in Moltona (panel A), Na Guardia (central B), and Es Curt (right C). Below growth curve, mean survival estimate of each stage by sex (males = black, females = grey). Bars indicate one standard deviation.

FIG. 4.—First and second components of the principal component analysis of 15 locus microsatellite genotypes from 80 insular lizards. Ovals are 95% confidence ellipses.

FIG. 5.—Coalescent trees used in Bayesian computation analysis. The scenarios assume 3 populations (Es Curt, Moltona, and Na Guardia) at present time. These populations diverged from a single population called ancestral population. Dashed lines in branches indicate the possibility of population size reduction. Change in pattern size of the branches indicates a

879 bottleneck. The time scale is on the right. On the bottom of each scenario shows posterior
880 probability of each one and its 95% confidence interval based on the logistic estimate.

881

Scenario 1

0.3127 (0.2936-0.3292)

Scenario 2

0.1223 (0.1126-0.1320)

Scenario 3

0.1015 (0.0925-0.1105)

Scenario 4

0.4635 (0.4466-0.4803)