Full title: The legacy of translocations among populations of the Ibizan Wall Lizard, *Podarcis pityusensis* (Squamata: Lacertidae)

Short title: Translocation among populations of *Podarcis pityusensis*

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Abstract

The Ibizan wall lizard, *Podarcis pityusensis,* was the subject of several documented translocations by the German herpetologist Martin Eisentraut, in 1930. He aimed to initiate long-term experiments into the evolution of melanism and other morphological traits and accordingly reported introductions into five islets that (he believed) contained no lizards. In this study, we analysed the genetics and morphological characteristics of individuals we found there. We found no lizards on two of the islets, namely Escull de Tramuntana or Galera, but for the first time, detected a large population on a third, Es Vaixell. Analyses of microsatellite DNA placed individuals from a fourth islet, Dau Gran, with those from the islet of Escull Vermell. They are also morphologically close to individuals from Escull Vermell. This suggests that selection pressures could have favoured the Escull Vermell phenotype following introduction. Eisentraut founded the Es Vaixell population with non- melanic Ibizan specimens, but the present day population of Es Vaixell was found to be fully melanic. Genetic markers support a strong similarity between Es Vaixell and Na Gorra, and indicate that, in all likelihood, the individuals introduced by Eisentraut have left no descendants. It is likely that Es Vaixell already contained lizards prior to this introduction. For reasons which we discuss, the translocations have revealed less than Eisentraut would have originally hoped for, although they do provide some potential insights into lizard morphological evolution following colonization.

Key-words: Balearic Islands - Ibiza - microsatellites - mtDNA

Introduction

Evolutionary changes can occur over months or decades in the wild (Kinnison, Unwin & Quinn, 2008). Unfortunately, there are a lack of empirical data on contemporary adaptive evolution. In general, morphological traits appear to be able to undergo rapid short-term changes after a population is exposed to new conditions (Kinnison & Hendry, 2001 for a review). These rapid evolutionary responses have been demonstrated in several organisms, from fish (Stockwell & Weeks, 1999) to mammals (Williams & Moore, 1989). Some key studies have also addressed this topic in reptiles (Kolbe *et al.*, 2008; Kolbe *et al.*, 2012; Thorpe, Reardon & Malhotra, 2005).

Field translocation experiments are employed as a useful tool to test predictions on fast evolutionary responses and directional selection intensity (Thorpe, Reardon & Malhotra, 2005 and references therein). In this study, instead of designing an experimental procedure to test some hypotheses, we analysed the results of experimental translocations made eighty-six years ago by the German herpetologist Martin Eisentraut (Eisentraut, 1949). Several voluntary or involuntary translocations among insular populations of lizards have been made in Balearic Islands over the years (Pérez-Mellado, 2009), but those made by Eisentraut were especially well documented and underpinned by a scientific objective which was to investigate the evolution of melanism and other morphological traits.

Eisentraut (1949) aimed to introduce individuals into islets that contained no lizards, but, unfortunately, it appears that some of these did in fact contain lizard populations. He was particularly interested in the adaptive values of these morphological characteristics for island lizards (Böhme, 2004). His first hypothesis was that melanism is selectively neutral and an indirect consequence of physiological changes related with dietary shifts in some insular populations (Eisentraut, 1949). It appears that Eisentraut was also interested in the heritability of these morphological characteristics and investigated this by introducing males and females from respective populations with very different morphologies into a new location. It should be pointed out that the experimental translocations were based on scientific knowledge in the 1930s and so Eistentraut's rationale can only be understood within this context (Böhme, 2004).

The model species employed by Eisentraut (1949) was *Podarcis pityusensis* (Boscá, 1883), a lacertid lizard from the western group of Balearic Islands, known as the Pityusic archipelago. It inhabits the main islands of Ibiza and Formentera, along with 42 of their associated islets (Pérez-Mellado, 2009), where it shows considerable phenotypic variation among populations, in terms of size, sexual dimorphism, scalation and coloration (including melanic populations). This has led to the recognition of 23 subspecies (Pérez-Mellado, 2009; Salvador & Pleguezuelos, 2002). We recently carried out a detailed analysis of its genetic diversity and historical biogeography and identified two main genetic groupings with some evidence of recent introgression between them (Rodríguez *et al*. 2013).

The five documented translocations (Eisentraut, 1930, 1949; Salvador, 1984) were as follows (the sex of the lizards was not documented unless stated below):

i) Eight melanic adult males from Escull Vermell (a small island off Western coast of Ibiza Island, Fig. 1) and 20 non-melanic adult females from Ibiza itself were introduced into the islet of Dau Gran (a small islet, located approximately 1.5 km from the Ibizan coast, Fig. 1).

ii) Non-melanic individuals (n= 24) were introduced from Ibiza into Escull de Tramuntana, (a small islet close to Escull Vermell, Fig. 1).

iii) Twenty non-melanic individuals were introduced from Ibiza into Galera, (a very small islet close to the northern coast of Vedra Island, Fig. 1).

iv) Fifty melanic lizards from Bleda Plana (a small island off western coast of Ibiza, Fig. 1) were introduced into Negra Llevant (an islet near Ibiza harbour, Fig. 1).

v) A similar number (n= 51) of non-melanic lizards from Ibiza Island were released in Es Vaixell, (a small islet close to Na Gorra Islet, Fig. 1).

Herpetologists made timely visits and observations in the years following these translocations. Lizards were observed during some visits to Dau Gran (experiment i) and Negra Llevant (experiment iv), although other visits seem to have not recorded lizards as being present. Lizards observed in Dau Gran were reported to have features from both founding populations. More specifically, their body dimensions and scalation were similar to melanic lizards from Escull Vermell, and their coloration/colour pattern was intermediate between Escull Vermell and Ibiza populations (non melanic) (Mayol, 2004). Böhme and Eisentraut (1981) also reported on the results of the Dau Gran translocation, reaching the conclusion that hybrids on the islet were larger than parental lizards, with increased variability in this new population compared with both parental populations. It appears that the present day population of Dau Gran is derived from the descendants of lizards introduced by Eisentraut in 1930, even if some author maintain that lizards from 1930 were extinct well before 1962, and that Dau Gran was later occupied by a more recent introduction in the early 1980s (Cirer, 1987). A very small population of Negra Llevant (experiment iv) were first recorded in 1979 (Cirer, 1987). Whether or not these were descendants of Eisentraut’s introductions is unknown although fishermen who had previously visited the island reported not having observed lizards there prior to this (Cirer, 1987).

The islets of Escull de Tramuntana (experiment ii), Galera (experiment iii) and Es Vaixell (experiment v) were visited during 1979 and 1980 as well as during 1962 and 1985 by J.P. Martínez-Rica (Cirer, 1987), but no lizards were observed in any of these islets. However, we have recently observed a population of large melanic individuals on Es Vaixell, which in part provided the motivation for the present analysis.

In summary, two of Eisentraut's introductions (Escull de Tramuntana and Galera) appear to have been unsuccessful (these islets may no longer contain lizards), but there are current lizard populations on Es Vaixell, Dau Gran and Negra Llevant islets, which may contain descendants of the introduced individuals. Here, we attempted to evaluate the results of Eisentraut's translocations by investigating the existence of lizard populations on these five islets and analysing the genetic and morphological characteristics of the extant populations that we found.

Material and methods

Specimens and populations

All lizards were captured by noosing and released after study at the site of capture. Tissue samples (tail tips) were obtained from 11 specimens from the islets that were subject to translocations: Es Vaixell, Dau Gran and Negra Llevant (2–5 from each). In addition, in the genetic analyses we included twenty individuals from Ibiza Main Island and ten islands/islets (Bleda Plana, Na Bosc, Escull Vermell, Na Gorra, Espartar, Escull d’Espartar, Espardell de s’Espartar, Bosc, Conillera and Ses Rates) from the western Pityusic archipelago, that is, from closely related populations to those under study (Rodríguez *et al.*, 2013, see Fig. 1 for exact location and Table 1).

Island/islet ecological characteristics were broadly described in terms of exposed areas, maximum altitudes and the number of species of vascular plants found there (Rivas, Costa & Loidi, 1992 and unpublished data, see Table 2).

DNA isolation and amplification

Total DNA was extracted using a standard phenol-chloroform protocol.

i) Microsatellites

Fifteen microsatellite loci, isolated from the closely-related *Podarcis lilfordi* (Bloor *et al.*, 2011), were genotyped in individuals from introduced populations. Primers, genotyping and allele assignment were the same as described for *P. lilfordi* in Bloor *et al.* (2011) and previously applied to *P. pityusensis* by Rodríguez *et al.* (2013). Previous genotypes established in *P. pityusensis* (Dryad Digital Repository. doi: 10.5061/dryad.r1538) for Ibiza and surrounding islands were included in the analyses (Rodríguez *et al*., 2013).

ii) Mitochondrial data

Five mtDNA fragments for each specimen were amplified and sequenced. The partial genes analysed were: 12S rRNA, cytochrome *b* (two regions), part of the control region and a fragment that included part of the ND1 gene, three tRNA genes ($tRNA_{Ile}$, $tRNA_{Gln}$, and $tRNA_{Met}$) and part of the ND2 gene (ND). Primers, amplification and sequenced conditions are the same as those used for *P. lilfordi* (Terrasa *et al*., 2009) and *P. pityusensis* (Rodríguez *et al*., 2013). Both heavy and light strands were aligned and visually checked using BioEdit ver.7.0.5.2 (Hall, 1999).

Previous sequences obtained for *P. pityusensis* (GenBank Accessions nos: EF694768, EF694794, JX852049–1, JX852053, JX852056–8, JX852063–7, JX852069–0, JX852076, JX852090–1, JX852093–4, JX852099, JX852101, JX852108, JX852118, JX852121–3, JX852125–6 and JX852130–1) and two *P. lilfordi* (GenBank Accessions nos: EF 694761, EF 694766, EF 694773, EF 694787, EF 694799, EF 694810, EF 990546, EU 006730 and EU 006756) were included in the analyses.

Microsatellite analysis

The number of genetically distinct clusters was estimated using STRUCTURE v.2.3.3 (Pritchard, Stephens & Donnelly, 2000) and STRUCTURE HARVESTER v.0.6.94 (Earl & vonHoldt, 2012). STRUCTURE was run twenty times using a model that incorporates admixture and correlated allele frequencies among populations, with each run comprising 400000 steps (200000 discarded as burn-in), for all values of K from 1–16. The programs STRUCTURE and CLUMPP v.1.1.2 (Jakobsson & Rosenberg, 2007) were used to determine the estimated membership coefficient (*Q*), and assign individuals to populations. We applied a threshold value of 0.20, as this provides a great efficiency and accuracy to differentiate between purebreds and hybrids (Vähä & Primer, 2006), so *Q* values around 0.2 and 0.8 may indicate hybridization between individuals from different clusters.

Mitochondrial analysis

Haplotypes were identified for the concatenated sequences and a haplotype network constructed using the program TCS v. 1.21 (Clement, Posada & Crandall, 2000). TCS creates a network using statistical parsimony (Templeton, Crandall & Sing, 1992; Templeton & Sing, 1993) that outputs the 95% plausible set of most parsimonious linkages among sequences. Bayesian inference of population structure was determined using BAPS v.5.3 (Corander, Waldmann & Sillanpää, 2003), with an upper bound of K=-20, and without prior information on geographic location.

An alternative phylogenetic analysis was used for comparison with the parsimony network. The mtDNA tree was obtained using Bayesian inference (BEAST v.1.8.) (Drummond *et al*., 2012), with model selection (test AICc) determined by jModelTest v.0.1.1 (Nylander *et al*., 2004). A lognormal relaxed clock model was used and a coalescent constant size, tree prior was specified. Two individuals of the sister species *P. lilfordi* from Dragonera and Aire islands were included as outgroups. Bayesian MCMC analyses were conducted with random starting trees, run for 50 million generations, and sampled every 1000 generations. Samples were analysed with Tracer v. 1.5 (Rambaut & Drummond, 2007) and TreeAnnotator (BEAST package) was used to combine and analyse the trees (trees were combined using the maximum sum of clade credibilities criterion).

Morphometric characters and analysis

The morphologies of 874 captured/museum specimens were also studied from photographs or measurements of live and preserved specimens (Table 1). All body dimensions were measured in the field, while scalation characters were recorded in the field or from digital images taken from each specimen. Additional specimens were studied from the Herpetological collections of the Alexander Koenig Museum of Natural History (Bonn, Germany), the Natural History Museum of London (UK), the Animal Biology Department of the University of Salamanca (Spain), the Zoology Department of the University of Barcelona (Spain) and the Herpetological Collection of the Natural History Museum of Madrid (Spain). Six body dimension characters were studied: snout-vent length (SVL), pileus length (PL), pileus width (PW), head height (HH), intact tail length (TL) and hind leg length (HLL). All measurements were made with a digital calliper to the nearest 0.01 mm, with the exception of SVL which was measured with a steel rule to the nearest 1 mm. Six scalation characters were studied: gularia (GUL), collaria (COL), dorsalia (DOR), ventralia (VEN), left femoralia (FEM) and left 4th digit lamellae (LAM). Not all characters could be recorded from all individuals (Supporting Information, Tables S1-S3).

Males and females were analysed separately, due to sexual dimorphism. Raw values were log-transformed for all characters to improve the fit to normality. We employed a Non-Metric Multidimensional Scaling (NMDS) to establish morphological divergence among 14 populations of *P. pityusensis*. The main advantage of NMDS is its suitability for nonlinear metric and even ordinal data. The method aims to depict the inherent pattern of a dissimilarity matrix in a geometric picture with a minimum number of dimensions while maintaining a close agreement with the initial data matrix (Clover, 1979). The dissimilarity matrices of Euclidean distances were constructed using the means of the log-transformed values of twelve morphometric characters (see above). We used the metaMDS function from the vegan R-package (Oksanen *et al.*, 2015). This function runs NMDS several times from random starting configurations, compares results and stops after detecting two similar minimum stress solutions. Finally, it scales and rotates the solution (Oksanen *et al.,* 2015). To assess the appropriateness of NMDS results we used a Shepard diagram to visualize the distances among populations in the ordination plot relative to the original distances. In addition, the goodness-of-fit of the ordination was assessed by the coefficients of determination (R^2) for the linear and non-linear regressions of the NMDS distances on the original ones (Borcard, Gillet & Legendre, 2011). Finally, we recorded the stress values of NMDS (Zuur, Ieno & Smith, 2007).

Results

Island/islet ecology

Ecological details are summarized in Table 2. Ibiza is a large and inhabited island and has the highest ecological complexity within the Pityusic archipelago in terms of microhabitat diversity, vegetation cover and predation pressure. Vegetation was extremely poor in all of the islets used for translocations by Eisentraut, even in comparison with islets next to the Western Ibizan coast (Table 2). The vegetation is comprised of only a few vascular plants that are strongly adapted to the extreme conditions of these isolated islets. No terrestrial predators are present in these islets (pers. obs.) while in Ibiza Island feral cats, genets and other lizard predators are common.

Dau Gran (experiment i)

A substantial lizard population was found on Dau Gran. Lizards from this population showed partial melanism, with dorsal colours being dark green and black. In almost half of adult individuals, we observed narrow dorsolateral lines, continuous or segmented. Dorsolateral lines can be dull golden or greenish. Ventrally, lizards are dark blue with green nuances. In some males, we observed rounded signal blue ocelli in outer ventral scales. The throat was profusely reticulated with signal black spots on a greenish or bluish background. Black spots are more or less aligned on submaxillary scales.

Escull de Tramuntana and Galera (experiments ii and iii)

We visited Escull de Tramuntana and Galera islets on spring 2013. Lizards were absent from both islets. Thus, we confirm previous reports (Cirer, 1987) and conclude that both of Eisentraut's introductions failed (ii and iii), probably because of a lack of trophic resources and suitable refuges (Table 2).

Negra Llevant (experiment iv)

We compared Negra Llevant lizards with the closest lizards from adjacent sites on Ibiza Island and lizards from Bleda Plana (the source of Eisentraut's translocated individuals). We found that adult males from Negra Llevant were significantly larger than those from Ibiza, but similar in size to those from Bleda Plana (one-way ANOVA of adult male SVL from the three populations: $F_{2, 198} = 35.64$, $p= 6.04 \times 10^{-14}$, see also Table S2). However, lizards from Negra Llevant showed quite similar patterns to those observed in several lizards from Ibiza Island and were completely different from the melanic lizards from Bleda Plana. The dorsal area of males and females were mostly green, with irregular black spots longitudinally arranged in a vertebral and two dorsolateral stripes. Flanks were greenish or brownish, spotted with black. Ventrally, lizards had bone-white or cream hues, in several cases with dark brown nuances and light blue eyespots on outer ventral scales.

Es Vaixell (experiment v)

Our first visit to Es Vaixell was in 2010 when we made the first observation of lizards on this islet. (This was followed by visits in 2013 and 2014). Relatively high population density was detected (allowing us to study over 65 individuals)..

Males and females were melanic, but we observed a light yellowish or dull golden nuance in the back, clearly contrasted with bluish flanks in 62.5% of individuals (instead of a uniform very dark blue or fully black colour). We did not observe any greenish dorsal coloration, as is common in lizards from neighbouring populations, such as those from Na Bosc islet. In almost all individuals, we observed narrow dorsolateral lines, continuous or segmented. Dorsolateral lines can be dull bluish or even yellowish or dull golden. In a few individuals we even observed reddish nuances in the anterior third of dorsolateral lines. Flanks are blackish spotted with cobalt blue rounded points. Ventrally, lizards are ultramarine blue with a large extension of areas of black blue, particularly, at the upper corners of ventral scales. Rounded signal blue ocelli in outer ventral scales were seen in some males, instead of greenish ocelli present in many lizards from Na Bosc and Na Gorra. The throat was profusely reticulated with signal black spots on an ultramarine blue background. The black colour of the throat is more intense and with better defined spots than in Na Gorra lizards. In addition, black spots are more or less aligned on submaxillary scales in lizards from Es Vaixell, but not in lizards from Na Gorra and Na Bosc.

Microsatellite DNA

Only nine individuals (five from Es Vaixell, and two from Negra Llevant and Dau Gran) were genotyped. The results, together with those from other studied populations (Rodríguez *et al.*, 2013), are shown in Table S3. In general, alleles are shared with other Ibizan populations, with some exceptions. The following private alleles were detected: allele 167 (Pli18) in both Dau Gran specimens and alleles 352, 404 (Pli4), 175 (Pli18) and 137 (Pli22) in some Es Vaixell individuals. Two alleles, 159 (Pli8) and 295 (Pli10) are only shared between Na Gorra and Es Vaixell, and the allele 246 (Pli12) is common between Es Vaixell and one specimen from Ibiza.

Two clusters were detected using the program Structure (ΔK 34.006) (Fig. 2A): the first (Cluster I) included samples from the introduced population of Es Vaixell and the second (Cluster II) contained the remaining samples, comprising lizards from Ibiza main island and surrounding islands, as well as the introduced populations of Dau Gran and Negra Llevant. In our analyses, we found one admixed individual from Na Gorra that presents a slightly lower membership coefficient (Q =0.725 to cluster II, Q =0.275 to cluster I, with samples from Es Vaixell) (Q values around 0.2–0.8 are indicative of hybridization between individuals from different clusters).

Mitochondrial DNA

The five mitochondrial fragments provide a total concatenated fragment length of 2383 bp (cytochrome b, 831 bp; 12S rRNA, 373 bp; ND1, 59 bp; ND2, 415 bp; tRNAs, 211 bp; control region 481 bp). New sequences have been deposited in GenBank (accession numbers KT002167–79). Sequences obtained from our previous studies were also used: GenBank accession numbers EF694768, EF694794, JX852058, JX852069–70, JX852076, JX852091, JX852093–4, JX852101, JX852121–3, JX852130–1.

The BAPS analysis defined three clusters (lnL= -757.5806, best visited partition) (Fig. 2B). The first cluster (A) includes samples from Ibiza, Na Gorra, Na Bosc, Bleda Plana, Es Vaixell, and one specimen from Dau Gran (Dg2). The second cluster (B) contains specimens from Bosc, Conillera, Ses Rates, Espartar, Espardell de s'Espartar, Escull d'Espartar, Na Bosc, Negra Llevant, Dau Gran, and one Ibiza sample. Specimens from Escull Vermell, Bleda Plana and Na Bosc constitute the third cluster (C). Hence, individuals from Ibiza Island (Clusters A, B), Na Bosc (Clusters A, B and C), Dau Gran (Clusters A and B) and Bleda Plana (Clusters A and C) are included in different clusters.

The TCS network (Fig. 3) revealed two main groups. The samples from Es Vaixell Island are found in one group only 0-2 mutational steps away from Na Gorra individuals. Two maternal haplotypes have been observed on Es Vaixell: one of them (present in four individuals) is shared with a specimen from Na Gorra, and the second one is separated by a unique mutational step from the first. Only 2 mutational steps or less separate the two remaining haplotypes from Na Gorra. MtDNA haplotypes from Dau Gran specimens are not shared by other insular populations, and evidence the existence of two highly differentiated lineages (>15 mutational steps); one of them (samples Dg1 and Dg3) is similar to Ses Rates and Na Bosc haplotypes, and the other (Dg2) is close to Ibiza main island specimens and Es Vaixell-Na Gorra haplotypes. The three mtDNA haplotypes from Negra Llevant are unique and are separated by three mutational steps from Espartar and by five from one specimen from Ibiza main island or a Na Bosc individual.

Finally, the Bayesian tree (Fig. 4) indicated two well-supported major clades. One of these clades contains Es Vaixell, Na Gorra, Ibiza Island, and some individuals from Dau Gran, Bleda Plana and Na Bosc. The second clade comprised the remaining individuals including individuals from Negra Llevant (which is close to individuals from Espartar). Although some Dau Gran individuals carry mtDNA that is very similar to that in Ibiza Island (Clade A), other individuals show similarities with Bosc and Ses Rates (Clade B). In contrast, specimens from Negra Llevant and Es Vaixell are only found in one clade.

Morphometric characteristics

In the case of non-metric multidimensional scaling, stress values in males and females (Fig. 5 and Fig. 6) were smaller than 0.05, indicating that both configurations were excellent and allowed for more detailed inspection (Zuur *et al*., 2007). The ordination of males (Fig. 5) and females (Fig. 5) was similar. Ibiza Island and the most remote populations to the west show the two most extreme (opposing) scores for the first NMDS coordinate, with islet populations closest to the coast of Ibiza showing intermediate values. In other words, Es Vaixell and Ibiza Island are found to be morphologically divergent. Negra Llevant was morphologically divergent from remaining populations for both males and females, including Bleda Plana. Finally, in the two-dimensional morphological space created by MDS analyses, Dau Gran is similar to Escull Vermell (the origin of the introduced males) and distant from Ibiza Island populations (the origin of introduced females) (Tables S2, S3).

Discussion

Negra Llevant individuals are genetically divergent from Bleda Plana: they do not correspond to the same mtDNA lineage, and the number of shared STR alleles between them is low. Moreover, their mtDNA clusters with specimens from the main island of Ibiza and other associated islets (see BAPS results, cluster B). Thus, we do not rule out additional introductions of lizards from Ibiza at an unknown date, or the existence of a population on the islet prior to Eisentraut's introduction (Cirer, 1987). On the other hand, lizards from Negra Llevant are morphologically similar in terms of body dimensions to those from Bleda Plana, the putative source population for Eisentraut’s translocation. This could suggest a genetic legacy of some Bleda Plana alleles being introduced at that time. However, the Negra Llevant population, in terms of pattern and colouration, shows greatest similarity with Ibiza main island populations also supporting the hypothesis that there has been an undocumented introduction of lizards from this island.

Two genetically well-differentiated mitochondrial lineages were detected in Dau Gran islet, both of which cluster with other Ibizan samples: the source of some of the lizards of Eisentraut's introduction. Similarities in microsatellite markers and morphology between individuals from Dau Gran and Escull Vermell individuals are explained by the fact that male lizards that Eisentraut introduced originated in Escull Vermell. Thus, we found evidence that the present day population of Dau Gran contains descendants of the two populations introduced by Eisentraut. However, lizards showed characteristics that most closely resemble one of the source populations suggesting that selection could have favoured the Escull Vermell over the Ibiza morphology on Dau Gran.

Although Zawadzki & Kroniger (2002) suggested that the population of Dau Gran consists of no more than 12–15 individuals, our results indicate a much larger population size. Zawadzki & Kroniger (2002) stressed the role of cannibalism and the strong intraspecific competition, with 85.7% of regenerated tails in lizards from this population. The high frequency of regenerated tails could indicate strong intraspecific competition, leading to strong selection pressures due to cannibalism (Mayol, 2004). This would predict a large body size with average SVLs that are greater than both parental populations. Böhme & Eisentraut (1981) found that the hybrids on this islet were larger than the parental lizards, and also showed increased variability. Salvador (1984) observed that lizards from Dau Gran showed morphological characteristics from two two populations: body size, robustness, body dimensions and meristic characters were similar to those from lizards from Escull Vermell while coloration was intermediate between Escull Vermell and Ibiza. He noted a partial melanism, especially in larger males. Our own results indicate that lizards from Dau Gran are closer to those of Escull Vermell (Fig. 5 and Fig. 6), with a partial melanism, large body size and similar scalation characters, both in males and females.

Lizards were not observed in Es Vaixell by previous authors (Cirer, 1987), probably because the highest elevations on this islet, covered with some vegetation (Table 2), are almost inaccessible without climbing equipment. The shared melanic coloration and genetic affinities between the Es Vaixell and Na Gorra populations are clearly indicative of their common ancestry. In contrast, genetic and morphological divergence between Es Vaixell lizards and those of Ibiza Island indicate that Eisentraut's introduction of lizards from Ibiza Island must have failed. Thus, contrary to Eisentraut's beliefs, a lizard population already existed on Es Vaixell. In addition, the genetic affinities between lizards from Es Vaixell and Na Gorra could indicate a relatively recent origin of Es Vaixell lizards from Na Gorra and, consequently, a rapid morphological change as a result of strong selection pressures in this tiny and very steep islet (Table 2). This could explain why lizards from Es Vaixell have a very large body size, both in males and females, a high number of subdigital lamellae in males, a low number of femoral pores (femoralia) and a large number of dorsal scales (Tables S2, S3).

The intensity of selection acting on translocated lizards was correlated with the magnitude of ecological change in the case of the Dominican lizard, *Anolis oculatus* (Malhotra & Thorpe, 1991; Thorpe, Reardon & Malhotra, 2005). We can expect that in our case, strong changes would be also observed in those translocations between populations with higher ecological differences. It is likely that the greatest differences in ecological conditions were between Ibiza Island and Es Vaixell or Dau Gran islets (Table 2). As a result, the Ibizan lizards introduced into Es Vaixell by Eisentraut disappeared without descendants. The remaining Es Vaixell individuals that we observe today originate from the original population, which Eisentraut (1949) did not know existed. Lizards from the large heterogenous island of Ibiza were unable to survive in the extreme environmental conditions on Es Vaixell (Table 2) and probably disappeared very quickly. They appear to have left no genetic legacy suggesting little or no successful interbreeding with the native population.

In contrast, the admixture of Escull Vermell and Ibiza Island lizards survived in Dau Gran, but the descendants of the original founders are morphologically closer to lizards from the remote Escull Vermell islet, which exhibits more similar ecological conditions to Dau Gran than to Ibiza Island (Table 2). The introduction of a limited number of individuals in a new population can be a sound strategy to learn about the respective contribution of founder effects and natural selection (Kolbe *et al.*, 2012) but, in some cases, as probably occurred in Es Vaixell, Galera and Escull de Tramuntana, the strong selection pressure overwhelmed the potential founder effects and the population perished.

In summary, the translocation experiments conducted by Eisentraut have not revealed much about the causes of morphological divergence in *P. pityusensis*. Some of them clearly failed, leaving no surviving populations today (Escull de Tramuntana and Galera). One translocation is difficult to interpret because the current population appears to contain lizards of mixed ancestry and is not easy to relate to Eisentraut's description (Negra Llevant). Another experiment appears to have involved the introduction of lizards to an islet that already contained a population, but fortunately, they appear to have made little or no contribution to the gene pool (Es Vaixell). Only the population on the islet of Dau Gran appears to be derived from Eisentraut's introductions.

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References

Bloor P, Rodríguez V, Terrasa B, Brown R, Pérez-Mellado V, Castro J, Picornell A, Ramon MM. 2011. Polymorphic microsatellite loci for the Balearic Island Lizard *Podarcis lilfordi* (Squamata: Lacertidae). *Conservation Genetic Resources* **3**:323-325.

Böhme W. 2004. the German contribution to Mediterranean Herpetology with special reference to the Balearic Islands and their lacertid lizards. In: Pérez-Mellado V, Riera, N & Perera, A, eds. *The biology of lacertid lizards: Evolutionary and ecological perspectives*. Maó, Menorca: Institut Menorquí d'Estudis, 63-82.

Böhme W, Eisentraut M. 1981. Vorläufiges Ergebnis eines unter natürlichen Bedingungen angesetzten Kreuzungversuchs bei Pityusen-Eidechsen, *Podarcis pityusensis* (Bosca, 1883) (Reptilia: Lacertidae). *Bonner Zoologische Beiträge* **32**: 145-155.

Borcard D, Gillet F, Legendre P. 2011. *Numerical Ecology with R*. New York: Springer.

Cirer AM. 1987. Revisión taxonómica de las subespecies del lacértido *Podarcis pityusensis*, Boscà, 1883. D. Phil. Thesis, Universitat de Barcelona.

Clement M, Posada D, Crandall K. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecoloy* **9**: 1657-1659.

Clover RC. 1979. Phenetic relationships among populations of *Podarcis sicula* and *P. melisellensis* (Sauria: Lacertidae) from islands in the Adriatic Sea. *Systematic Biology* **28**: 284-298.

Corander J, Waldmann P, Sillanpää MJ. 2003. Bayesian analysis of genetic differentiation between populations. *Genetics* **163**: 367-374.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969-1973.

Earl D, vonHoldt B. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetic Resources* **4**: 359-361.

Eisentraut M. 1930. Beitrag zur Eidechsenfauna der Pityusen und Columbreten. *Mitteilungen aus dem Zoologischen Museum in Berlin* **16**: 397-410.

Eisentraut M. 1949. Die Eidechsen der Spanischen Mittelmeerinseln und ihre Rassenaufspaltung im Lichte der Evolution. *Mitteilungen aus dem Zoologischen Museum in Berlin* **26**: 1-225.

Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleid Acids Symposium Series* **41**: 95-98.

Jakobsson M, Rosenberg NA. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801-1806.

Kinnison MT, Hendry AP. 2001. The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* **112-113**: 145-164.

Kinnison MT, Unwin MJ, Quinn TP. 2008. Eco-evolutionary vs. habitat contributions to invasion in salmon: experimental evaluation in the wild. *Molecular Ecology* **17**: 405-414.

Kolbe JJ, Larson A, Losos JB, de Queiroz K. 2008. Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species. *Biology Letters* **4**: 434-437.

Kolbe JJ, Leal M, Schoener TW, Spiller DA, Losos JB. 2012. Founder effects persist despite adaptive differentiation: a field experiment with lizards. *Science* **335**: 1086-1089.

Malhotra A, Thorpe RS. 1991. Experimental detection of rapid evolutionary response in natural lizard populations. *Nature* **353**: 347-348.

Mayol J. 2004. Survival of an artificially hybridized population of *Podarcis pityusensis* at Dau Gran: evolutionary implications. In: Pérez-Mellado V, Riera, N & Perera, A, eds. *The biology of lacertid lizards: Evolutionary and ecological perspectives*. Maó, Menorca: Institut Menorquí d'Estudis, 239-244.

Nylander JA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL. 2004. Bayesian phylogenetic analysis of combined data. Systematic Biology **53**: 47-67.

Oksanen J, Guillaume Blanchet F, Kindt R, Legendre O, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2015. Community Ecology Package. R package version 2.2-1. Available at: http://CRAN.R-project.org/package=vegan2015.

Pérez-Mellado V. 2009. *Les sargantanes de les Balears*. Palma de Mallorca: Edicions Documenta Balear.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959.

Rambaut A, Drummond AJ. 2007. Tracer v. 1.5. Available from http://tree.bio.ed.ac.uk/software/tracer/.

Rivas S, Costa M, Loidi JJ. 1992. La vegetación de las islas de Ibiza y Formentera (Islas Baleares, España). *Itinera geobotanica* **6**: 99-236.

Rodríguez V, Brown RP, Terrasa B, Pérez-Mellado V, Castro JA, Picornell A, Ramon MM. 2013. Multilocus genetic diversity and historical biogeography of the endemic wall lizard from Ibiza and Formentera, *Podarcis pityusensis* (Squamata: Lacertidae). *Molecular Ecology* **22**: 4829-4841.

Salvador A. 1984. A taxonomic study of the Eivissa wall lizard, *Podarcis pityusensis* Boscà 1883. In: Kuhbier H, Alcover JA, Guerau d' Arellano Tur C, eds. *Biogeography and ecology of the Pityusic Islands*. The Netherlands: Dr W. Junk Publisher, 393-427.

Salvador A, Pleguezuelos JM. 2002. *Reptiles españoles: identificación, historia natural y distribución*. Talavera de la Reina: Canseco Editores.

Stockwell CA, Weeks SC. 1999. Translocations and rapid evolutionary responses in recently stablished populations of western mosquitofish (*Gambusia affinis*). *Animal Conservation* **2**: 103-110.

Templeton AR, Crandall KA, Sing CF. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**: 619-633.

Templeton AR, Sing CF. 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. *Genetics* **134**: 659-669.

Terrasa B, Pérez-Mellado V, Brown RP, Picornell A, Castro JA, Ramon MM. 2009. Foundations for conservation of intraspecific genetic diversity revealed by analysis of phylogeographical structure in the endangered endemic lizard *Podarcis lilfordi*. *Diversity and Distributions* **15**: 207-221.

Thorpe RS, Reardon JT, Malhotra A. 2005. Common garden and natural selection experiments support ecotypic differentiation in the Dominican anole (*Anolis oculatus*). *The American Naturalist* **165**: 495-504.

Vähä J-P, Primmer CR. 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology* **15**: 63-72.

Williams CK, Moore RJ. 1989. Phenotypic adaptation and natural selection in the rabbit, *Oryctolagus cuniculus*, in Australia. *Journal of Animal Ecology*, **58**: 495-507.

Zawadzki M, Kroniger M. 2002. Eidechsenbeobachtungen auf Dado Grande: Ergänzende Bemerkungen zu einem unter natürlichen Bedingungen angesetzten Kreuzungsversuch bei Pityusen-Eidechsen *Podarcis pityusensis* (Boscà, 1883). *Die Eidechse* **13**: 33-42.

Zuur AF, Ieno EN, Smith GM. 2007. *Analysing Ecological Data*. New York: Springer.

Figure 1. Map of the Pityusic archipelago (Balearic Islands), sample localities are indicated.

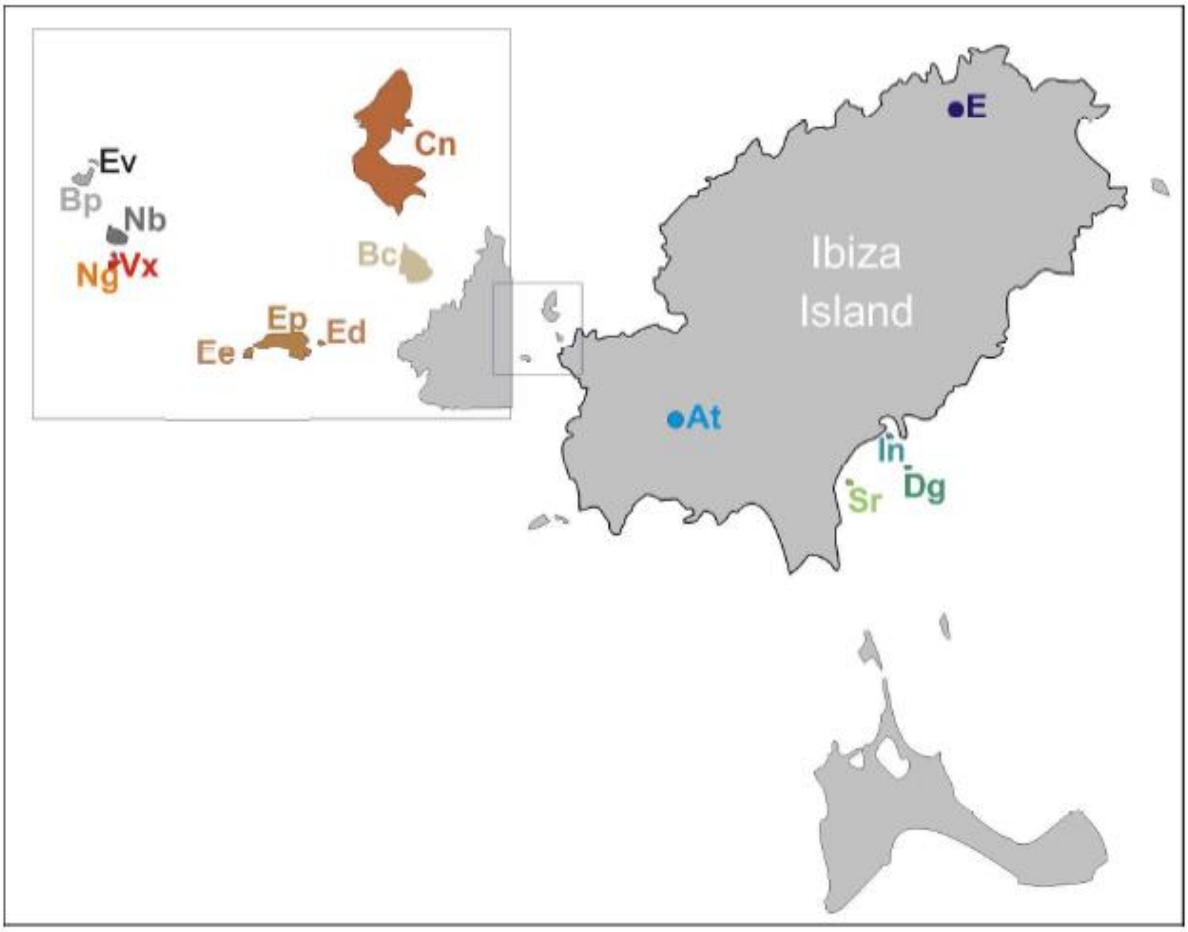

Figure 2. Genetic structure inferred from microsatellite DNA and mtDNA. A) Microsatellite-based Bayesian assignments identified two main groups (cluster I: green and cluster II: red). The bar plot shows estimated membership coefficient (***Q***); vertical bars represent individuals and assignment probabilities. B) Mixture analyses for mitochondrial data, estimated by BAPS software, identified three clusters (cluster A: green, cluster B: red and cluster C: blue). In the Bar plot, vertical bars represent individuals and proportions of admixture, with different colours corresponding to different ancestral sources.

A) Microsatellite data

B) Mitochondrial data

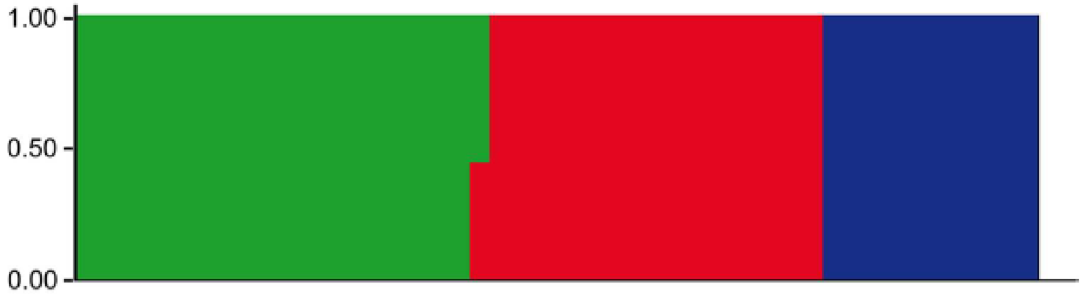

Figure 3. MtDNA haplotype network. Specimens from each locality are represented by individual colours.

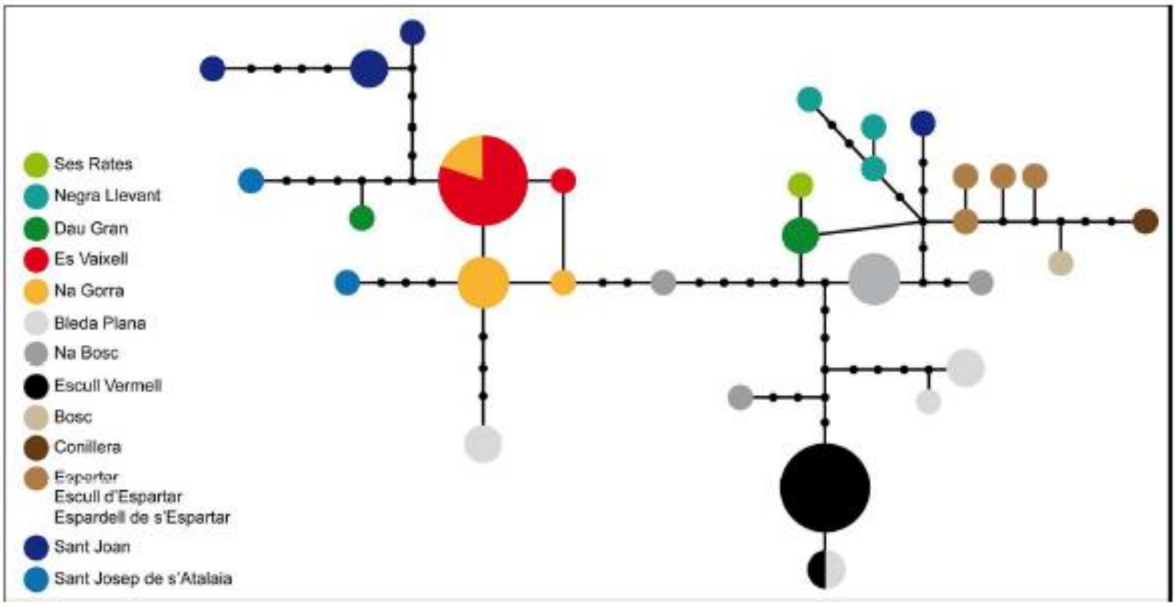

Figure 4. Mitochondrial phylogenetic tree estimated by BEAST showing relationships among *P. pityusensis* haplotypes. Bayesian posterior probabilities (> 0.9) are indicated on the tree.

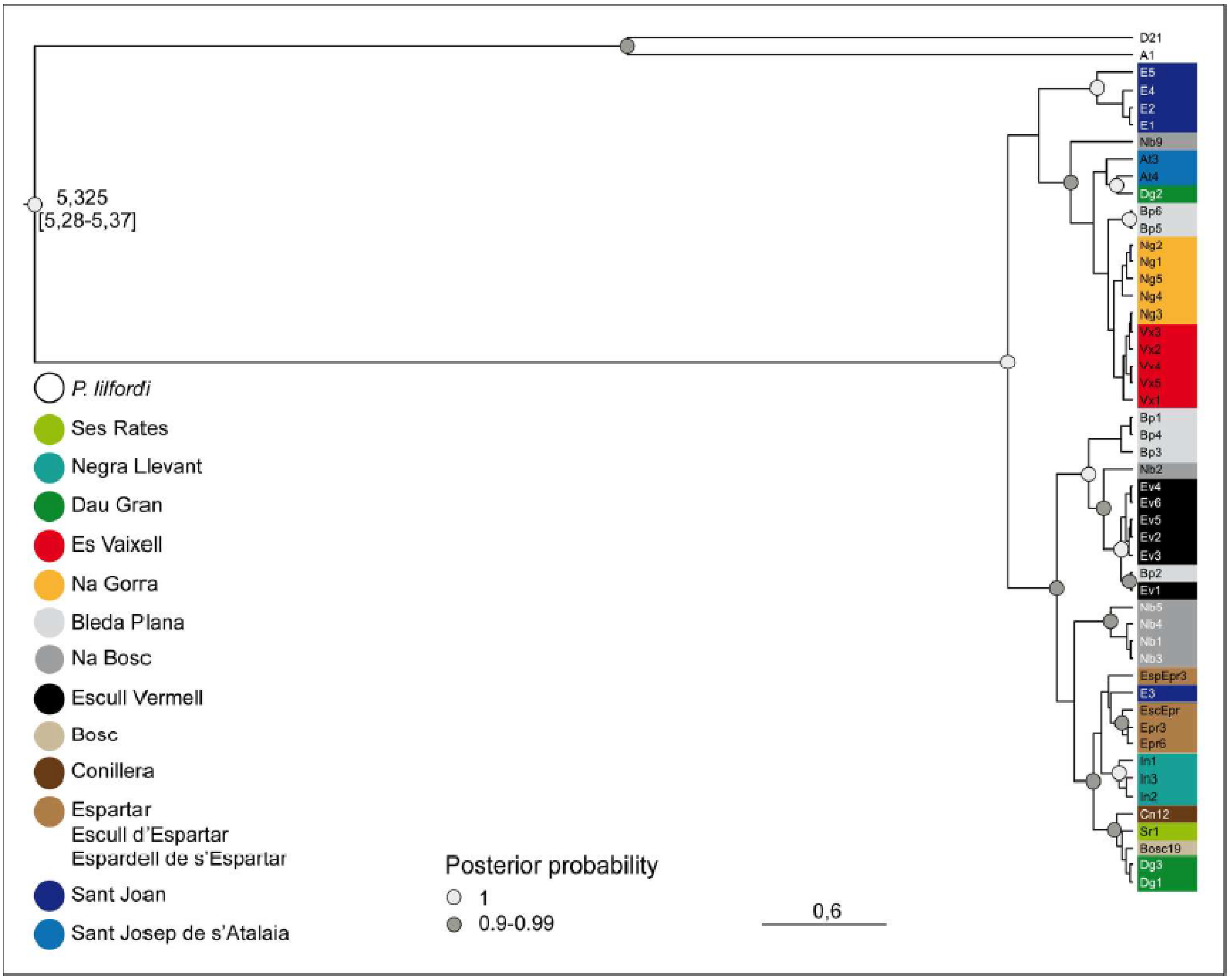

Figure 5. Non-Metric multidimensional scaling of adult males

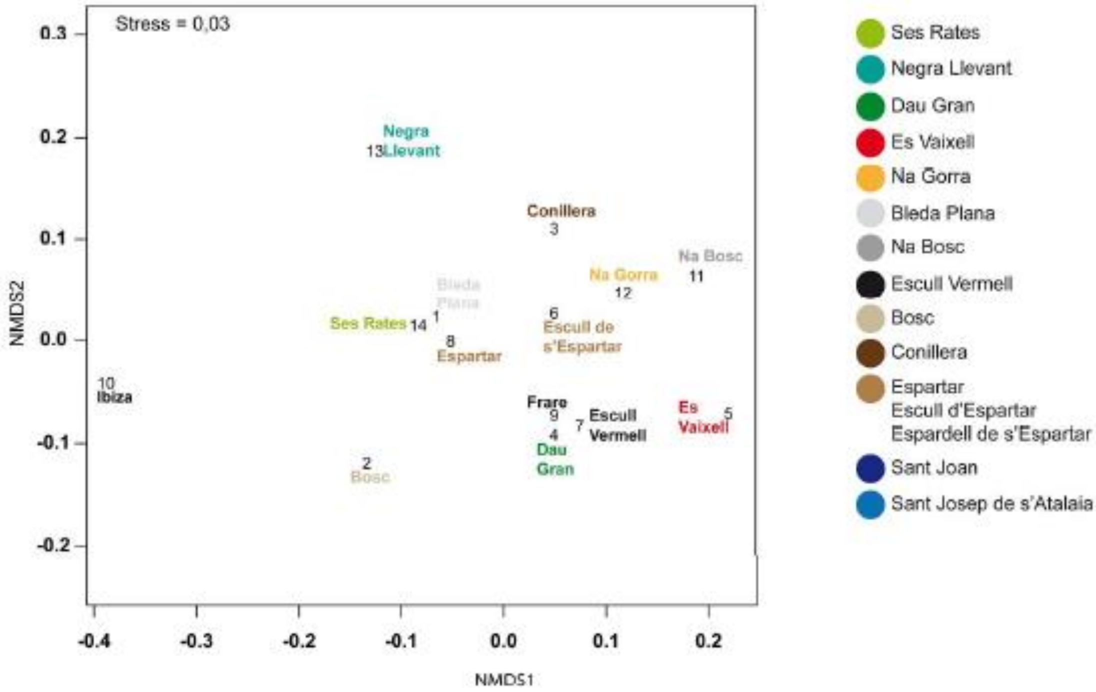

Figure 6. Non-Metric multidimensional scaling of adult females

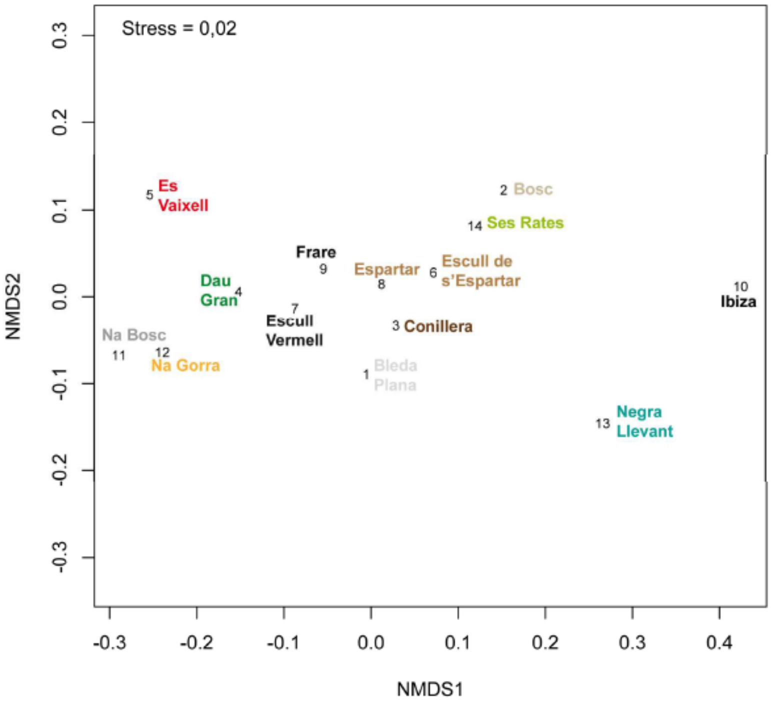

Table 1. Localities and number of specimens (N) analysed for DNA (microsatellites and mitochondrial DNA) and for morphometric characters (males and females columns) from *P. pityusensis*. Introduced populations are indicated in bold. Lizards sampled from Ibiza Island originated from 11 different localities (see Supplementary Material).

n	**Id**	**Locality**	**DNA**	**Morphometric characters**	
				Males	**Females**
		Islands surrounding Ibiza			
1	Bp	Bleda Plana	1-6	99	82
2	Nb	Na Bosc	1-6	31	27
3	Ev	Escull Vermell	1-6	32	24
4	Ng	Na Gorra	1-5	49	33
5	**Vx**	**Es Vaixell**	5	27	27
6	Ep	Espartar	2	61	36
7	Ed	Escull d'Espartar	1	7	12
8	Ee	Espardell de s'Espartar	1	19	26
9	Bc	Bosc	1	23	19
10	Cn	Conillera	1	44	28
11	Sr	Ses Rates	1	12	11
12	**Dg**	**Dau Gran**	3	21	34
13	**In**	**Negra Llevant**	3	24	4
		Ibiza Island			
14		11 localities		78	39
14a	E	Sant Joan	5		
14b	At	Sant Josep de s'Atalaia	1-2		
		TOTAL	48	472	402

Table 2. Ecological characteristics of the localities under study. Introduced populations are indicated in bold. Lizards sampled from Ibiza Island originated from 11 different localities (see Supplementary Material). (There is no Id entry for the islets of Galera and Escull because there are not represented in Fig 1).

n	Id	Locality	Island area (ha)	Maximum altitude (m)	Number of vascular plants
1	Bp	Bleda Plana	3.12	18	13
2	Nb	Na Bosc	3.12	39	31
3	Ev	Escull Vermell	0.04	10	7
4	Ng	Na Gorra	1.56	29	11
5	**Vx**	**Es Vaixell**	0.03	20	6
6	Ep	Espartar	18.75	70	131
7	Ed	Escull d’Espartar	0.08	20	6
8	Ee	Espardell de s’Espartar	0.56	20	17
9	Bc	Bosc	18.12	66	98
10	Cn	Conillera	100	66	139
11	Sr	Ses Rates	1.00	12	58
12	**Dg**	**Dau Gran**	0.02	12	1
13	**In**	**Negra Llevant**	0.37	9.5	16
14		Ibiza Island	57,260	475	921
15		Galera	0.19	6	4
16		Escull de Tramuntana	0.04	10	1