

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

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The Ibizan wall lizard, *Podarcis pityusensis*, was the subject of several documented translocations by the German vertebrate zoologist Martin Eisentraut, in 1930. He aimed to initiate long-term experiments into the evolution of melanism and other morphological traits and accordingly he designed introductions into five islets that (he believed) contained no lizards. In this study, we analyzed the genetic and morphological characteristics of individuals we found there. We found no lizards on two of the islets, namely Escull de Tramuntana and Galera, but for the first time, detected a large population on a third islet, Es Vaixell. Eisentraut founded the Es Vaixell population with non-melanistic Ibizan specimens, but the present day population of Es Vaixell was found to be fully melanistic. Genetic markers support a strong similarity between Es Vaixell and its neighbour islet, 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. Analyses of microsatellite DNA placed individuals from a fourth islet, Dau Gran, with those of one of its source islet, 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. For reasons we discuss, the translocations have revealed less than Eisentraut would have originally hoped for. Although evolutionary processes are normally time-consuming, these translocations do provide some potential insights into the rapid evolution of lizard morphology following colonization.

ADDITIONAL KEYWORDS: Balearic Islands – Ibiza – microsatellites – mtDNA.

INTRODUCTION

Evolutionary changes can occur over months or decades in the wild (Kinnison, Unwin & Quinn, 2008) but there is a lack of empirical data on contemporary adaptive evolution. In general, morphological traits appear 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 (Herrel *et al.*, 2008; Kolbe *et al.*, 2008, 2012; Stuart *et al.*, 2014; 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 *et al.*, 2005 and references therein). In this study, instead of designing an experimental procedure to test some hypotheses, we analyzed the results of some translocations made 86 years ago by the German vertebrate zoologist Martin Eisentraut (Eisentraut, 1949). These translocations were especially well documented and

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underpinned by a scientific objective which was to investigate the evolution of melanism and other morphological traits (Böhme, 2004). His first hypothesis was that melanism is selectively neutral and an indirect consequence of physiological changes related to 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 populations with very different morphologies into a new location. It should be pointed out that these experimental translocations were based on scientific knowledge in the 1930s and so Eisentraut'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 surrounding islets (Pérez-Mellado, 2009), where it shows considerable phenotypic variation among populations, in terms of size, sexual dimorphism, scalation, and coloration (including melanistic populations).

Eisentraut (1949) aimed to introduce individuals into islets that contained no lizards, but, unfortunately, it now appears that some of these did in fact contain lizard populations. The five documented translocations (Eisentraut, 1930, 1949; Salvador, 1984) were as follows (the sex of the lizards was not documented unless stated below. Within parentheses, we give the names of the islets employed by Eisentraut, 1949, see Fig. 1 for the location of source populations, the number of individuals employed in the translocation, and the target populations).

Translocation (i). Eight melanistic adult males from Escull Vermell (Escui de Vermey) and 20 non-melanistic adult females from Ibiza itself were introduced into the islet of Dau Gran (Dado Grande, Fig. 1).

Translocation (ii). Non-melanistic individuals ($n = 24$) were introduced from Ibiza into Escull de Tramuntana (Escui de Tremontana).

Translocation (iii). Twenty non-melanistic individuals were introduced from Ibiza into Galera islet.

Translocation (iv). Fifty melanistic lizards from Bleda Plana (Fig. 1) were introduced into Negra Llevant (Negra del Este, Fig. 1).

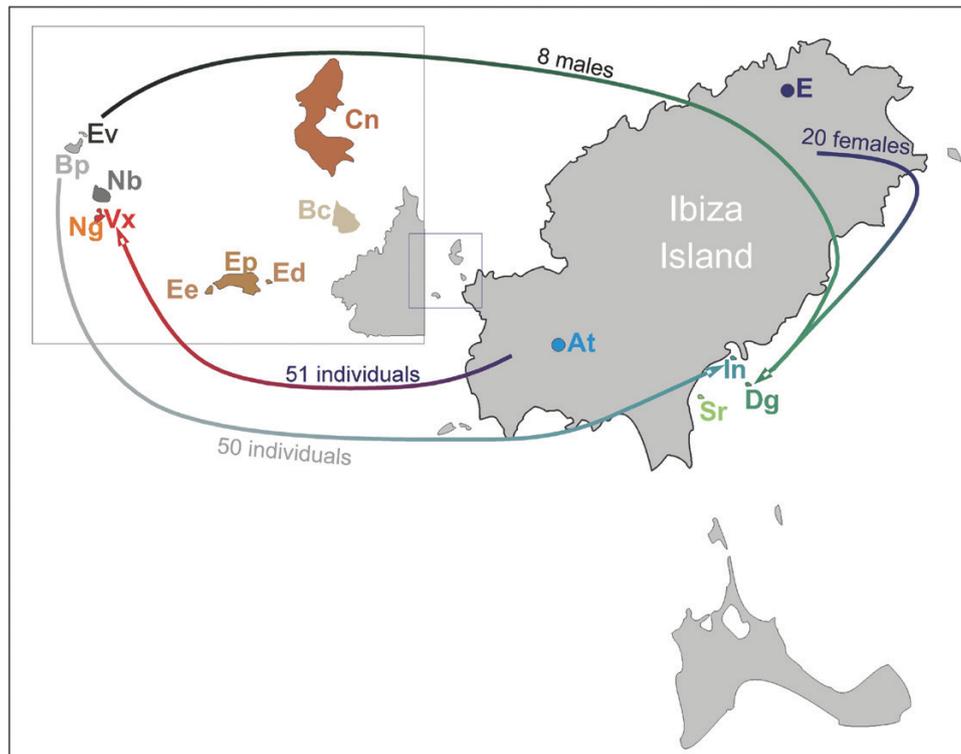


Figure 1. Map of the Pityusic archipelago (Balearic Islands) and translocations made by Eisentraut in 1930. From Ibiza Island: E: Sant Joan, At: Sant Josep de s'Atalaia. Islands surrounding Ibiza: Bp: Bleda Plana, Nb: Na Bosc, Ev: Escull Vermell, Ng: Na Gorra, Vx: es Vaixell, Ep: Espartar, Ed: Escull d'Espartar, Ee: Espardell de s'Espartar, Bc: Bosc, Cn: Conillera, Sr: Ses Rates, Dg: Dau Gran, Ln: Negra Llevant. Arrows indicate the origin and destination of each translocation, as well as the number of lizards involved (see also Table 1).

Translocation (v). Fifty-one non-melanistic lizards from Ibiza Island were released in Es Vaixell (Veschell de Gorra, Fig. 1).

Herpetologists made timely visits and observations in the years following these translocations. Lizards were observed during some visits to Dau Gran (see dates in [Böhme and Eisentraut, 1981](#), translocation i; [Mayol, 2004](#)) and Negra Llevant (translocation iv). Lizards observed in Dau Gran were reported to have features from both founding populations ([Böhme and Eisentraut, 1981](#); [Mayol, 2004](#)). A very small population of Negra Llevant (translocation iv) was first recorded in 1979 ([Cirer, 1987](#)). The islets of Escull de Tramuntana (translocation ii), Galera (translocation iii), and Es Vaixell (translocation 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.

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. We looked for the existence

of lizard populations on these five islets and analyzed 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 (two to five from each). In addition, in the genetic analyses we included 20 individuals from Main Island of Ibiza and 10 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](#)).

Table 1. Localities and number of specimens analyzed for DNA (microsatellites and mitochondrial DNA) and for morphometric characters (males and females columns) from *Podarcis pityusensis*. Introduced populations are indicated in bold. Populations are numbered (*n*) as in [Figs 6](#) and [7](#). Lizards sampled from Ibiza Island originated from 11 different localities (see Supporting Information).

<i>n</i>	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 Supporting Information). (There is no Id entry for the islets of Galera and Escull de Tramuntana, because they are not represented in Fig. 1). Populations are numbered (n) as in Figs 6 and 7.

<i>n</i>	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

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 analyzed 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 numbers: EF694768, EF694794,

JX852049–51, JX852069–70 JX852076, JX852090–1, JX852093–4, JX852099, JX852101, JX852108, JX852118, JX852121–3, JX852125–6 and JX852130–1) and two *P. lilfordi* (GenBank accessions numbers: EF694761, EF694766, EF694773, EF694787, EF694799, EF694810, EF990546, EU006730 and EU006756) 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 20 times using a model that incorporates admixture and correlated allele frequencies among populations, with each run comprising 400 000 steps (200 000 discarded as burn-in), for all values of *K* from 1 to 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 & Primmer, 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 analyzed with Tracer v.1.5 (Rambaut & Drummond, 2007) and TreeAnnotator (BEAST package) was used to combine and analyze 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, pileus width, head height, intact tail length, and hind leg length. 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, collaria, dorsalia, ventralia, left femoralia, and left fourth digit lamellae. Not all characters could be recorded from all individuals (Tables S1–S3).

Males and females were analyzed 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 non-linear 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 12 morphometric characters. 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 (personal observation), while in Ibiza Island feral cats, genets, and other lizard predators are common.

DAU GRAN (TRANSLOCATION I)

A substantial lizard population was found on Dau Gran. Lizards from this population showed partial melanism, with dorsal colors 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 broadly aligned on submaxillary scales (Fig. 2B).

ESCULL DE TRAMUNTANA AND GALERA (TRANSLOCATIONS 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 (ii and iii) failed, because of a lack of suitable refuges or other unknown factors.

NEGRA LLEVANT (TRANSLOCATION IV)

We compared Negra Llevant lizards (Fig. 2C) with the closest lizards from adjacent sites on Ibiza Island (Fig. 2E) and lizards from Bleda Plana (Fig. 2A), 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 melanistic 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 (TRANSLOCATION 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 melanistic (Fig. 2F), but we observed a light yellowish or dull golden nuance in the back, which clearly contrasted with bluish flanks in 62.5% of individuals (instead of a uniform very dark blue or fully black color). 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, 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 color 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 (Fig. 2F).

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 S4. In general, alleles are shared with other Ibiza 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. 3A): 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, and JX852130–1.

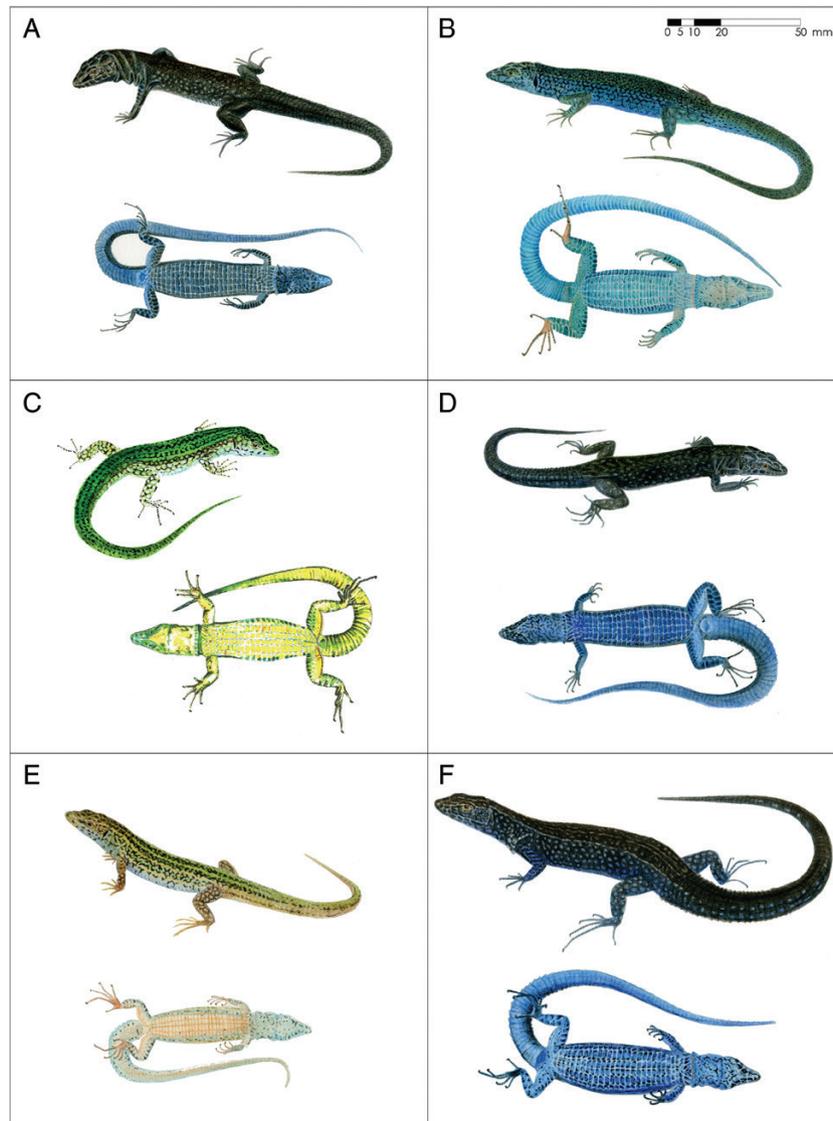


Figure 2. Dorsal and ventral views of male lizards from populations involved in translocations made by Eisentraut. (A) Bleda Plana islet. Lizards are dorsally melanistic in this population, with blue bellies and flanks spotted with grey marks. (B) Dau gran islet. Lizards are dorsally dark blue with a clear tendency to melanism. Ventral parts are blue and sparsely spotted with darker marks. (C) Negra Llevant islet. Lizards from this islet are dorsally green, spotted with large black marks and stripes. The upper parts of flanks are a mixture of brown areas spotted with greenish and yellowish marks. Bellies are yellowish, with blue and green spots, especially in outer ventral scales. (D) Escull Vermell islet. Lizards from this islet are dorsally melanistic, with only small lighter spots in the flanks. Ventrally, lizards are dark blue with black marks covering the entire surface of the throat. (E) Ibiza Island. Lizards are extremely variable, but most of them have dorsally aligned black spots or stripes on a green background. Bellies are cream or slightly orange with small black and bluish spots. (F) Es Vaixell islet. Lizards from this islet are dorsally melanistic, with dark blue spots in the flanks and very dark blue bellies (see more details in the text).

The BAPS analysis defined three clusters (lnL = -757.5806, best visited partition) (Fig. 3B). 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 des'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

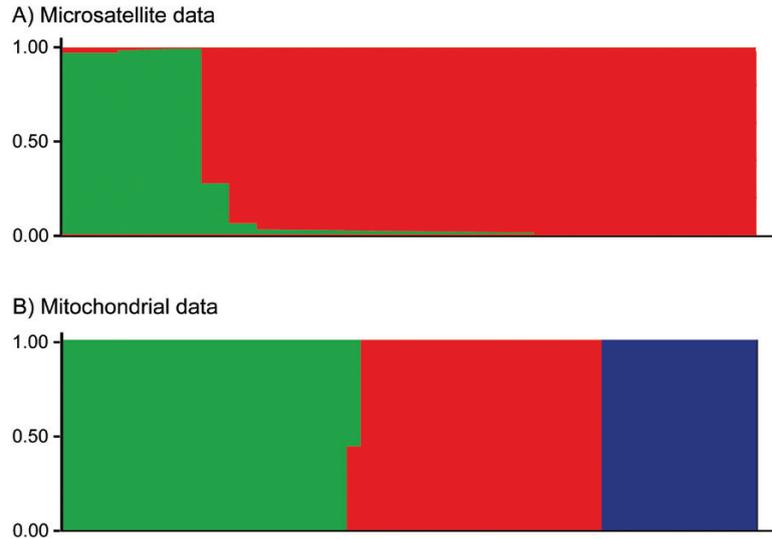


Figure 3. Genetic structure inferred from microsatellite DNA and mtDNA. (A) Microsatellite-based Bayesian assignments identified two main groups (Cluster I, green and cluster II, red). The bar plot shows estimated membership coefficient (Q); vertical bars represent individuals and assignment probabilities. (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 colors corresponding to different ancestral sources.

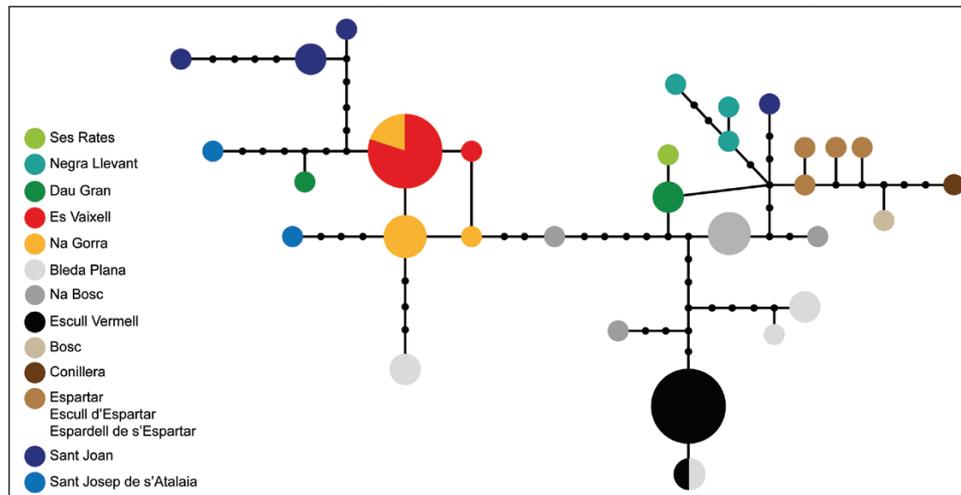


Figure 4. MtDNA haplotype network. Specimens from each locality are represented by individual colors.

Island (Clusters A and 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. 4) revealed two main groups. The samples from Es Vaixell Island are found in one group only zero to two 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 two 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.

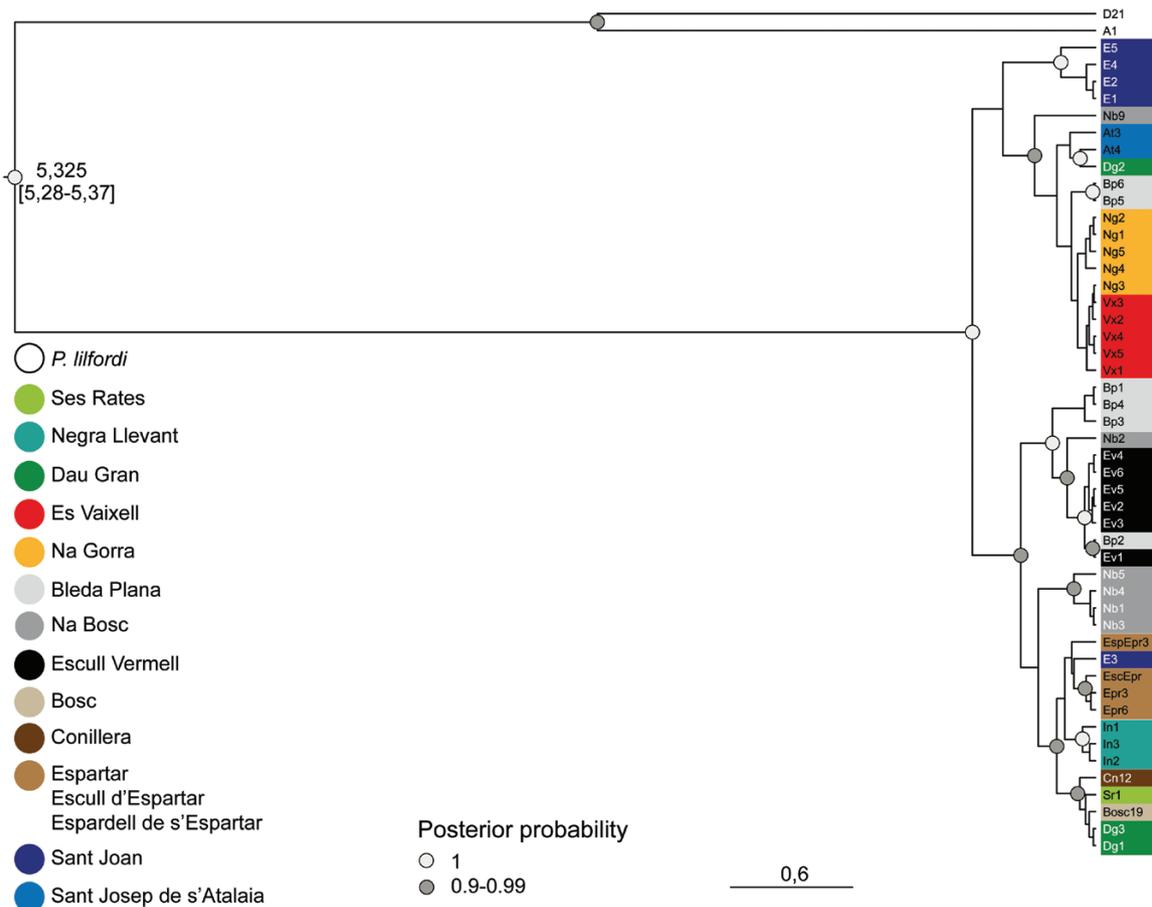


Figure 5. Mitochondrial phylogenetic tree estimated by BEAST showing relationships among *Podarcis pityusensis* haplotypes. Bayesian posterior probabilities (>0.9) are indicated on the tree.

Finally, the Bayesian tree (Fig. 5) 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 (Figs 6 and 7) 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. 6) and females (Fig. 7) 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 and 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). From the time of Eisentraut's translocations, other

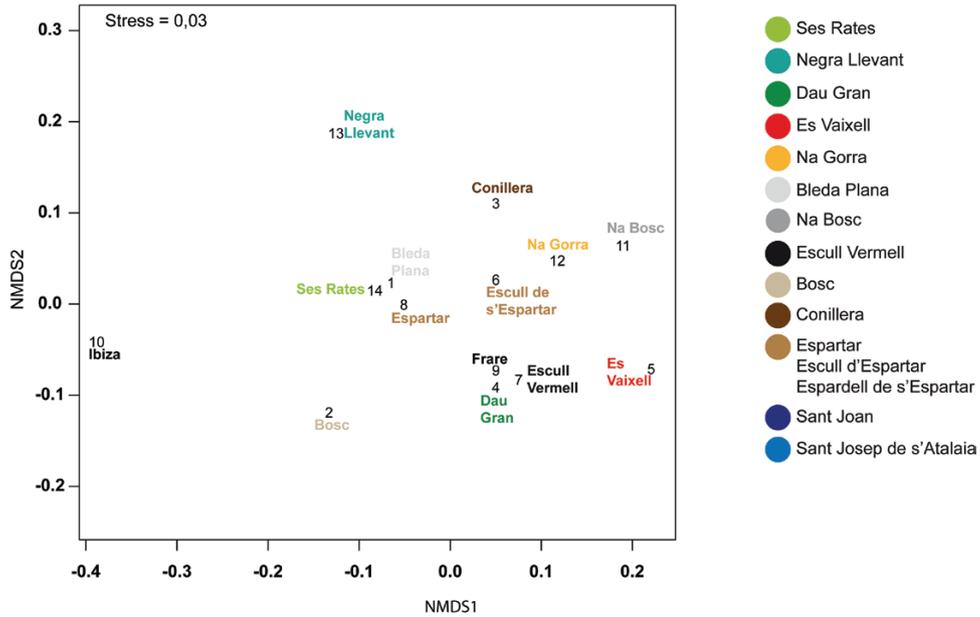


Figure 6. Non-metric multidimensional scaling of adult males (populations are numbered as in Tables 1 and 2).

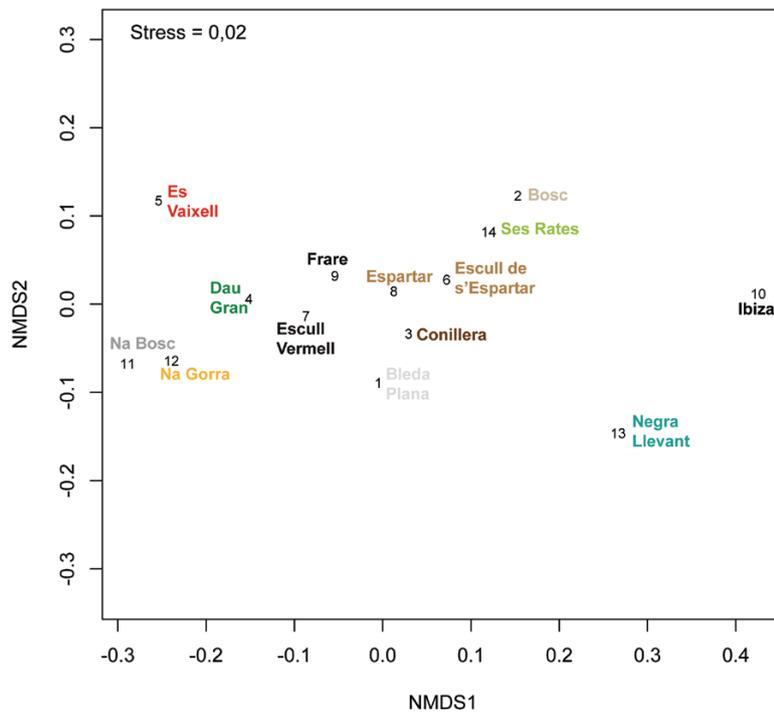


Figure 7. Non-metric multidimensional scaling of adult females (populations are numbered as in Tables 1 and 2).

accidental arrivals of lizards to coastal islets could take place. Thus, we cannot 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 coloration, 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. The majority of older and remote islets of Balearics and other Mediterranean archipelagos house lizards of larger body sizes and melanistic coloration, as those from Escull Vermell (see, e.g., Pérez-Mellado, 2009). Thus, we can speculate that these morphometric characteristics are adaptive in these remote, small, and old islets.

Although Zawadzki & Kroniger (2002) suggested that the population of Dau Gran consists of no more than 12–15 individuals, our observations 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 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 (Figs 6 and 7), with a partial melanism, large body size, and similar scalation characters, both in males and in 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 base of Es Vaixell lacks vegetation cover and is frequently whipped by sea waves. The shared melanistic 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 in females, a high number of subdigital lamellae in males, a low number of femoral pores (femorals), and a large number of dorsal scales (Tables S2 and 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 *et al.*, 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 originated from the original population, which Eisentraut (1949) did not know existed. Lizards from the large heterogeneous 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 revealed that not all lizards coming from different populations were able to survive in islets to which they had been translocated. They did not have the same potential for survival of these new environmental conditions. 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. Even the negative results of some of the experiments are of interest, as they raise questions about the factors driving the success of colonization in these species of lacertid lizards.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Morphometry of *Podarcis pityusensis* specimens under study.

Table S2. Morphometric characters of adult males.

Table S3. Morphometric characters of adult females.

Table S4. Microsatellite data.