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## Ecological divergence combined with ancient allopatry in lizard populations from a small volcanic island

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Complete List of Authors:	Suarez, Nicolas; Universidad de Las Palmas de Gran Canaria, Departamento de Genética Pestano, Jose; Universidad de Las Palmas de Gran Canaria, Departamento de Genética Brown, Richard; Liverpool John Moores University, School of Natural Sciences & Psycholog
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1 **Ecological divergence combined with ancient allopatry in lizard populations from a small**  
2 **volcanic island**

3 N. M. Suárez<sup>1</sup>, J. Pestano<sup>1</sup> and R. P. Brown<sup>2†</sup>

4 <sup>1</sup>Departamento de Genética, Facultad de Medicina, Universidad de Las Palmas de Gran Canaria,  
5 Las Palmas 35080, Gran Canaria, Spain.

6 <sup>2</sup>School of Natural Sciences & Psychology, Liverpool John Moores University, Liverpool L3  
7 3AF, UK.

8 †Correspondence

9

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13 **Correspondence author information:**

14 Richard P. Brown: School of Natural Sciences & Psychology, Liverpool John Moores  
15 University, Liverpool L3 3AF, UK; Tel: (+44) 151 231-2159: [r.p.brown@ljmu.ac.uk](mailto:r.p.brown@ljmu.ac.uk)

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18 **Running Title:** Ecological and allopatric divergence within an island

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**23 Abstract**

24 Population divergence and speciation are often explained by geographical isolation, but may also  
25 be possible under high gene flow due to strong ecology-related differences in selection pressures.  
26 This study combines coalescent analyses of genetic data (11 microsatellite loci and 1 Kbp of  
27 mtDNA) and ecological modelling to examine the relative contributions of isolation and ecology  
28 to incipient speciation in the scincid lizard *Chalcides sexlineatus* within the volcanic island of  
29 Gran Canaria. Bayesian multispecies coalescent dating of within-island genetic divergence of  
30 northern and southern populations showed correspondence with the timing of volcanic activity in  
31 the north of the island 1.5-3.0 Ma ago. Coalescent estimates of demographic changes reveal  
32 historical increases in the size of northern populations, consistent with expansions from a  
33 volcanic refuge. Nevertheless, ecological divergence is also supported. First, species distribution  
34 modelling shows that the northern morph is associated with mesic habitat types and the southern  
35 morph with xeric habitat types. It seems likely that the colour morphs are associated with  
36 different anti-predator strategies in the different habitats. Second, coalescent estimation of gene  
37 copy migration (based on microsatellites and mtDNA) suggest high rates from northern to  
38 southern morphs demonstrating the strength of ecology-mediated selection pressures that  
39 maintain the divergent southern morph. Together, these findings underline the complexity of the  
40 speciation process by providing evidence for the combined effects of ecological divergence and  
41 ancient divergence in allopatry.

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## 47 Introduction

48 Geographical isolation has traditionally been considered the main driving force behind  
49 population divergence (Mayr 1963). However, there has been considerable recent interest in  
50 ecological speciation, the process by which selection pressures promote speciation despite high  
51 gene flow (Rundle et al., 2000; Rundle and Nosil 2005; Egan et al. 2008). Under this model,  
52 divergent regions within the genome can arise due to reproductive incompatibilities or strong  
53 selection. Greatly reduced gene flow is expected in these regions, compared with higher gene  
54 flow in neutral regions (Hey 2006). Evidence for these patterns is starting to emerge (e.g., Nosil  
55 et al. 2012). Nevertheless, it may often be over-simplistic to assume that ranges and habitats have  
56 remained the same for long periods of time and that populations have diverged *in situ*. Historic  
57 geographical interruptions to gene flow may have played a role in shaping current patterns,  
58 although it is often difficult to demonstrate the combined effects of isolation and ecology (but  
59 see Thorpe et al. 1996; Thorpe and Richard 2001; Wang et al., 2013). Identification of good  
60 ecological models will help reveal new insights into the complex interplay of past and present  
61 gene flow and selection on the speciation process (Cowie and Holland 2006; Heaney 2007;  
62 Schilthuizen et al. 2011; Strasburg and Rieseberg 2011).

63 The Canary Island archipelago is of volcanic origin and located in the eastern Atlantic  
64 Ocean, off NW Africa. The scincid lizard *Chalcides sexlineatus* is endemic to the central island  
65 of Gran Canaria which it appears to have colonized at the beginning of the Pliocene or earlier  
66 (Brown and Pestano 1998). The island is only 1532 km<sup>2</sup> but reaches an altitude of 1949 m and  
67 shows strong zonation of habitat (Figure 1). Trade winds blow onto the north-facing slopes of  
68 Gran Canaria throughout the year causing relief rainfall. The north slopes are consequently more  
69 densely vegetated and were once home to laurisilva forest. In contrast, the southern slopes  
70 experience warmer, more arid conditions with low cloud and sparse vegetation. Two different

71 morphs of *C. sexlineatus* have been described and largely correspond to these two areas (Brown  
72 and Thorpe 1991a,b; Brown et al. 1991). The northern (N) morph has a relatively uniform brown  
73 dorsum and orange ventrum, while the southern (S) morph tends to have a black dorsum with  
74 light stripes and bright blue tail (Brown and Thorpe 1991b; Brown et al. 1991). The morphs also  
75 differ substantially in body dimensions and scalation (Brown and Thorpe 1991a). The transition  
76 between the two morphs is quite sharp but populations with intermediate morphologies are  
77 present in these regions. The correlation between morphology and habitat type, and the finding  
78 of a similar habitat-morphology association on a neighbouring island initially led Brown et al.  
79 (1991) to propose that different selection regimes had led to morphological divergence.

80         Geographical structuring of mtDNA is strongly N-S within Gran Canaria and concordant  
81 with the morphological variation (Pestano and Brown 1999). Timing of mtDNA divergence  
82 appears to coincide with the last major eruptive cycle on Gran Canaria, which began about 3 Ma,  
83 and covered large parts of the NE of the island (Carracedo 2011). This lends greater support to  
84 the hypothesis that the two morphological forms had originated in isolated volcanic refugia  
85 (although it should be pointed out that relatively short slowly-evolving mtDNA sequences were  
86 analysed and relationships were not fully resolved) (Pestano and Brown 1999).

87         While these studies have pointed to the effects of both historical isolation and natural  
88 selection, more detailed investigation is required. To achieve this we analyse: i) more  
89 informative mtDNA sequence from a larger number of individuals than analysed previously and  
90 ii) previously identified microsatellite markers (Suarez et al. 2008) to investigate divergence  
91 across the nuclear genome. Coalescent-based methods are employed to estimate levels of gene  
92 flow between populations and more rigorously date the timing of population divergence with the  
93 aim of understanding how such large morphological differences could arise within such a small

94 island. We also use species distribution modelling to investigate whether distributions predicted  
95 from biotic and abiotic features of the environment are distinct for the different morphotypes,  
96 allowing further evaluation of the ecological speciation hypothesis.

97

## 98 **Materials and methods**

### 99 *Samples*

100 A total of 650 *C. sexlineatus* were captured by hand from 26 evenly distributed sample sites  
101 covering the entire distribution of the species between October, 2001 and July, 2002 (Figure 1  
102 and Supplementary Table S1). Tail-tips were removed, placed in 100% ethanol, and individuals  
103 released at the site of capture. Genomic DNA was extracted using the PureGene DNA  
104 Purification Kit (Gentra) following the manufacturer's instructions. DNA from other Canary  
105 Island *Chalcides* was also available from previous projects: 16 *C. viridamus* from Tenerife,  
106 representing the 3 main lineages within that island (Brown et al. 2000), 6 *C. coeruleopunctatus*  
107 from the islands of El Hierro (3 individuals) and La Gomera (3 individuals) and 2 *C. simonyi*  
108 from Lanzarote.

109 Ethics statement: Field permits were granted by the Consejería de Medio Ambiente, Cabildo  
110 Insular de Gran Canaria.

111

### 112 *Mitochondrial DNA amplification, sequencing and characterization*

113 MtDNA sequences were obtained from a subsample of 137 *C. sexlineatus*, representing all  
114 sample sites within Gran Canaria (Figure 1), and also from all other Canary Island *Chalcides*  
115 from which DNA was available. A single 997-999 bp mtDNA fragment was amplified. It  
116 contained partial sequences from the NADH dehydrogenase gene subunits 1 (ND1) and 2 (ND2)

117 and three intervening tRNAs (tRNA<sup>Ile</sup>, tRNA<sup>Gln</sup> and tRNA<sup>Met</sup>). Polymerase chain reactions  
118 (PCRs) were carried out in 25 µl volumes using: 20–50 ng DNA, 1X buffer (Bioline; 16 mM  
119 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 67 mM Tris–HCl (pH 8.8) and 0.01% Tween 20), 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each  
120 dNTP, 0.2 U of Taq DNA polymerase (Bioline) and 1 µM of each primer. Primers used by  
121 previous studies were applied to all specimens, except for those from sites 19 and 38 in Gran  
122 Canaria for which new primers were designed (see Supplementary Table S2; Macey et al. 1997,  
123 Macey et al. 1998). PCR conditions were as follows: 94°C for 3 min, followed by 30 cycles at  
124 94°C for 1 min, 55°C for 1 min and 72°C for 2 min with a final extension at 72°C for 10 min,  
125 performed in a GeneAmp<sup>®</sup> PCR System 2700 (Applied Biosystems). PCR products were  
126 purified using MicroSpin<sup>™</sup> S-400 HR Columns (GE Healthcare) and sequenced on an ABI  
127 PRISM 3130XL automatic sequencer (Applied Biosystems). Chromatograms were checked by  
128 eye for ambiguities and sequences were edited and aligned using ClustalW within BioEdit ver.  
129 7.1.3.0 (Hall 1999). Estimation of genetic diversity (i.e., nucleotide and haplotype diversity) and  
130 tests of neutrality (Tajima's D [(Tajima 1989)] and Fu's F<sub>s</sub> [(Fu 1997)]) were carried out using  
131 DnaSP ver. 5.10.1 (Librado and Rozas 2009).

132

### 133 *Intraspecific mtDNA tree*

134 The *C. sexlineatus* mtDNA tree was estimated using the Bayesian inference approach  
135 implemented within MRBAYES v3.1.2 (Ronquist and Huelsenbeck 2003). Sequences were  
136 partitioned into the following functional sets: 1) 1<sup>st</sup> codon positions (ND1+ND2), 2) 2<sup>nd</sup> codon  
137 positions (ND1+ND2), 3) 3<sup>rd</sup> codon positions (ND1+ND2), and 4) tRNAs. MRMODELTEST  
138 ver. 2.3 (Nylander 2004) was used to test models of molecular evolution for each partition by  
139 analyses of their log-likelihoods using the Akaike Information Criterion (AIC). Two independent

140 MRBAYES analyses were run from different starting points for  $2 \times 10^6$  steps and the results  
141 compared. Each run comprised four chains, with genealogies being sampled every 100 steps.  
142 MCMC performance was assessed by examination of convergence of posteriors using TRACER  
143 ver. 1.5 (Rambaut and Drummond 2007).  $4 \times 10^5$  steps were discarded as burnin. A 50% majority  
144 rule consensus tree was constructed from the post-burnin posterior tree sample from one of the  
145 runs.

146

#### 147 *Population divergence times and demographic changes*

148 The multispecies coalescent method implemented in \*BEAST ver. 1.7.4 (Heled and Drummond  
149 2010, Drummond et al. 2012) was applied to the mtDNA with the aim of estimating time of  
150 divergence between groups within Gran Canaria using a well-established external calibration.  
151 This approach takes ancestral polymorphism into account. Species units within the analysis were  
152 generally represented by divergent lineages rather than by formally-recognized species and so  
153 will be referred to as “population groups”. All described Canary Island specimens were used in  
154 the analysis. Ten population groups were defined, with two or more sequences available for each  
155 group (recognized species in parentheses): i) N Gran Canaria (*C. sexlineatus*), ii) SE Gran  
156 Canaria (*C. sexlineatus*), iii) W Gran Canaria (*C. sexlineatus*), iv) S Gran Canaria (*C.*  
157 *sexlineatus*), v) NE Tenerife (*C. viridanus*), vi) NW Tenerife (*C. viridanus*), vii) Central Tenerife  
158 (*C. viridanus*), viii) La Gomera (*C. coeruleopunctatus*), ix) El Hierro (*C. coeruleopunctatus*), x)  
159 Lanzarote (*C. simonyi*).

160 Following previous findings, monophyly constraints were applied to: i) all Gran Canaria  
161 population groups, ii) N, SE, and W Gran Canaria groups, iii) all Tenerife groups, iv) La Gomera  
162 and El Hierro groups, v) all Gran Canaria, Tenerife, La Gomera, and El Hierro population groups



163 (see Brown and Pestano 1998; Carranza et al. 2008). A Yule prior was used to specify  
164 divergence times across the tree. The prior on the divergence time for the (El Hierro, La Gomera)  
165 node was specified from the Gamma distribution  $G(12.5, 2.0)$ , where the respective values are  
166 the shape and scale parameters, but with hard minimum and maximum limits of (0, 1.12). This  
167 provided increasing density between 0 and 1.12, reflecting prior knowledge that El Hierro was  
168 colonized from La Gomera soon after its emergence 1.12 Ma (this is supported by the degree of  
169 sequence divergence described by Brown and Pestano 1998). A prior hard maximum bound of  
170 11.6 Ma was placed on the node that was most basal to all population groups from *C.*  
171 *sexlineatus*, *C. viridanus*, and *C. coeruleopunctatus*. This corresponded to the age of the second  
172 oldest of the islands on which they are found (Tenerife). The rationale for this prior is that at  
173 least two emerged islands must have been present to allow dispersal-mediated speciation.  
174 Finally, a maximum bound of 20.6 Ma was placed on the root node. This represents the time of  
175 appearance of the first (eastern) Canary Island, and appears to considerably predate the  
176 divergence time of the *Chalcides* group containing *C. simonyi* from the other Canary Island  
177 *Chalcides* (which has been previously estimated at around 7 Ma; Carranza et al. 2008).

178 Sequences were partitioned into: i) codons 1 and 2, ii) codon 3, and iii) tRNAs. The  
179 HKY+G model of substitution was applied to each partition and a lognormal uncorrelated rates  
180 relaxed clock model used. The *Chalcides* tree was quite shallow which can have the effect of  
181 making some priors (such as the prior on times) quite influential, in the absence of suitable prior  
182 knowledge, particularly under a relaxed clock (Brown and Yang 2010; Brown and Yang 2011).  
183 Hence, the results were compared with those from a strict clock analysis. MCMC chains were  
184 run for  $4 \times 10^7$  cycles sampled at intervals of 2000, providing 20000 samples from the posterior,  
185 of which the first 2000 were discarded as burnin, leaving 18000 samples for analysis.

186 Historical demographic changes in the four Gran Canarian population groups were  
187 analysed using Bayesian skyline plots (BSPs) under the piecewise-constant model (Drummond  
188 et al. 2005). Priors on rates of the four partitions were specified using normal distributions, the  
189 means and variances of which were derived from the posterior distributions of rates from the  
190 dating analyses. The BSP approach requires user-specification of the number of groups of  
191 coalescent intervals (this reduces potential noise associated with a large number of short  
192 intervals: Drummond et al. 2005). We specified 4 groups, but results were similar when larger  
193 numbers of groups (up to 10) were tested.

194

#### 195 *Nuclear DNA amplification, genotyping and characterization*

196 Eleven autosomal microsatellite loci were analysed for all 650 individuals. All loci contained  
197 tetranucleotide (AAAG) repeats. We use the same locus names and multiplex PCR protocol  
198 described previously (Suarez et al. 2008). Genotyping was performed on an ABI PRISM  
199 3130XL genetic analyser (Applied Biosystems) with G5 matrix and GeneScan-500 (LIZ) as size  
200 standard. Alleles were scored using GeneMapper v4.0 software (Applied Biosystems). Measures  
201 of genetic diversity and other statistics were obtained using ARLEQUIN version 3.11 (Excoffier  
202 et al. 2005) and FSTAT 2.9.3 (Goudet 1995).

203

#### 204 *Genetic structure (microsatellite DNA)*

205 Genetic structuring of nuclear DNA was inferred by application of the model-based clustering  
206 method implemented in the program STRUCTURE ver.2.3.4 (Pritchard et al. 2000) to all 650  
207 specimens. An admixture model with correlated allele frequencies among populations was  
208 applied. Twenty STRUCTURE runs (chain length =  $10^6$  steps, burn-in =  $10^5$ ) were performed for

209 different numbers of genetic clusters ( $K$ ) between 1 and 10 (see Gilbert et al. 2012). We used  
210 STRUCTURE HARVESTER web version 0.6.92 (Earl and vonHoldt 2012) to analyse the output  
211 using the  $\Delta K$  metric approach proposed by Evanno et al. (2005). This provides an objective and  
212 therefore preferable alternative to simply selecting  $K$  according to the magnitude of its log-  
213 likelihood (which may lead to overestimation of the number of genetic clusters; Evanno et al.  
214 2005). Prior information on the origin of each sampled individual was not used in the analysis.  
215 CLUMPP (Jakobsson and Rosenberg 2007) was used to concatenate the data from the multiple  
216 runs for each  $K$  and assign individuals to clusters using their membership coefficient ( $Q$ ). A  
217 threshold value of  $Q = 0.2$  was used because it is efficient and accurate at differentiating between  
218 purebreds and hybrids (Vaha and Primmer 2006).

219

#### 220 *Analysis of migration and isolation*

221 Estimation of timing of divergence and migration between the two main morphotypes within  
222 Gran Canaria was carried out using the coalescent method implemented within IMA2 (Hey and  
223 Nielsen 2007; Hey 2010). Sampled locations were assigned to either N or S morphs according to  
224 geographical position relative to the midpoint of the morphological variation that has been  
225 described previously (see Figure 1, Brown and Thorpe 1991b and Brown et al. 1991 for more  
226 details). All 137 mtDNA sequences (60 N and 77 S morphs) and 50 microsatellite genotypes (25  
227 N and 25 S morphs with representatives from all 26 sites, for all 11 loci), were analysed. The  
228 microsatellite data had to be subsampled in this way because several months were required to run  
229 the MCMC chains for the complete data set.

230 The HKY model of DNA substitution (Hasegawa et al. 1985) was used for the mtDNA  
231 fragment, and the stepwise mutation model (SMM; Kimura and Ohta 1978) was used for the

232 microsatellites. Following preliminary runs using diffuse priors, tighter uniform priors were  
233 specified:  $U(0,6)$  on divergence time,  $U(0,300)$  on population sizes and  $U(0,1)$  on migration  
234 rates. Consistency of results of results was compared between three replicate runs starting from  
235 different positions. A final definitive MCMC chain was run for  $1.01 \times 10^8$  steps, with parameters  
236 sampled every 100 steps, and the first  $1 \times 10^6$  steps discarded as burnin.

237         In order to convert the estimates into more interpretable demographic units, a generation  
238 time of 2 years was used (derived from personal observations and evidence that similar species  
239 from cooler regions in northern Spain reach sexual maturity within 2-3 years: Galan 2003). A  
240 mutation rate of  $1.8351 \times 10^{-5}$  mutations/locus/year for the entire mtDNA sequence was  
241 determined from the \*BEAST analysis of divergence times. IMA2 provides migration rates  
242 looking backwards in time, but here we present the results in the more intuitive forward direction  
243 of time.

244

#### 245 *Species distribution modelling and spatial analyses*

246 Species distribution models (SDMs) were constructed separately for the N and S morphs using  
247 the maximum entropy algorithm implemented in MAXENT ver. 3.3.3 (Phillips et al. 2006). We  
248 used the coordinates of the 46 sample localities (25 N sites and 21 S sites) in Brown and Thorpe  
249 1991a,b as evidence of presence (Supplementary Table S3). Note that the sample sites used here  
250 are a subset of these 46 sites.

251         The environment was modelled from a subset of 56 climatic layers obtained from the  
252 WorldClim global climate database (<http://www.worldclim.org>). The climatic layers had a  
253 spatial resolution of 30 arc-seconds (ca.  $1 \text{ Km}^2$ ). A categorical variable representing potential  
254 vegetation was obtained from land characterisation maps published by the Canary Island

255 Government (<http://visor.grafcan.es/visorweb/>). Seventeen vegetation categories were used with  
256 presence of each vegetation type being recorded for each sample square using the viewer tool (30  
257 arc-seconds grid) provided on the database (Supplementary Figure 1).

258         There was no prior biological evidence to support objective determination of suitable  
259 climate predictors in the MAXENT analyses. In addition, most climate predictors were  
260 correlated. We therefore used two approaches to select climatic variables: 1) after preliminary  
261 runs using all 56 climate variables, a subset of 6 variables was determined according to  
262 permutation importance which is an indirect estimator of the dependence of the model on the  
263 selected variable (see MAXENT documentation and Supplementary Table S3), 2) just two  
264 uncorrelated climate variables were selected: precipitation seasonality (which also had high  
265 permutation importance) and temperature seasonality. In both analyses, the selected climatic  
266 variables were combined with potential vegetation and the results compared.

267         The N and S morph SDMs were tested for statistical significance by comparison of the  
268 observed area under the curve (AUC) for the receiver operating characteristic (ROC) plot with  
269 the same AUCs obtained by random sampling of the same number of sample squares (Raes and  
270 ter Steege 2007). This null model approach prevents interpretation of model quality using an  
271 arbitrary AUC threshold and removes the need to set aside samples for model testing.  
272 Randomized point data were created with ENMTools ver. 1.3 (Warren et al. 2010). A total of  
273 500 AUCs were generated (including the observed AUC). Statistical significance was established  
274 when the magnitude of the observed AUC was equal or greater than the value of the 475<sup>th</sup> rank-  
275 ordered AUC (corresponding to  $P \leq 0.05$ ).

276         We examined niche overlap using a principal components analysis (PCA) on the two  
277 subsets of climatic variables. Schoener's *D* index was used to test for niche overlap between N

278 and S morphs, with its significance being tested using a randomization test (see Warren et al.,  
279 2008).  $D$  can take values from 0 (no overlap) to 1 (complete overlap). Significance of  $D$  was  
280 achieved by comparison with 100 datasets containing random partitions of N and S occurrences.

281

## 282 **Results**

### 283 *MtDNA diversity and phylogeography*

284 Lengths of genes/partial genes that were sequenced were as follows: ND1, 307 bp; tRNAs, 215-  
285 217 bp (tRNA<sup>Ile</sup>, 77-79 bp; tRNA<sup>Gln</sup>, 71 bp; tRNA<sup>Met</sup>, 67 bp); ND2, 475 bp (GeneBank accession  
286 numbers: KJ463905-KJ464030). One hundred and seven haplotypes were detected within *C.*  
287 *sexlineatus*, with polymorphisms at 234 sites (Supplementary Table S4).

288 The four main mitochondrial lineages were designated as N, S, SE and W according to  
289 their distributions within Gran Canaria (Figure 2). The basal node representing divergence  
290 between the (N, SE, W) and S lineages was strongly supported. Most sample sites provided  
291 individuals from a single mtDNA lineage although two or three lineages were identified at three  
292 sites (site 14: S and E lineages detected, site 27: N and SE lineages, and site 10: N, S and SE  
293 lineages).

294 Nucleotide diversity was lowest in the SE mtDNA lineage and highest in the W lineage  
295 (Table 1). In the N lineage, there was a significant deviation from neutral expectation for  
296 Tajima's  $D$  and Fu's  $F_s$ , a small Rozas'  $R^2$  and a significant signal in sequence mismatch  
297 distributions, consistent with recent expansion/dispersal (Table 1). BSPs provided evidence of  
298 two substantial increases in population size in this lineage, one of which was during the last 50  
299 ka. Evidence of less-pronounced increases in population sizes of the S and SE lineages was also  
300 detected by the BSPs (Figure 3).

301

302 *Population divergence times*

303 A likelihood ratio test was used to compare the likelihoods of mtDNA trees with and without a  
304 constant rate assumption (HKY+G model) and revealed significant violation of the clock  
305 ( $2\Delta l=460.19$ ,  $P<0.0001$ ). However, the \*BEAST posterior median divergence time for the basal  
306 Gran Canaria node was similar under the strict (2.00 Ma [95% HPD: 0.88-3.59 Ma]) and the  
307 relaxed clock analyses (1.94 Ma [0.86-3.46]) (Figure 4). This was the case for all other nodes,  
308 such as the root (strict clock: 7.97 Ma [3.93-14.02], relaxed clock: 7.77 Ma [3.76-13.67]). Hence,  
309 only relaxed clock estimates will be discussed from here onwards.

310

311 *Population genetic analysis of microsatellite loci*

312 Microsatellite polymorphism was high: the number of alleles per locus ranged from 23 (locus  
313 *Csex11*) to 38 (locus *Csex01*), with a mean of 28.6 (site summary statistics are in Supplementary  
314 Table S5). There was significant deviation from HWE for some loci within populations, but there  
315 was no clear pattern across localities. There was significant LD between some pairs of loci (after  
316 Bonferroni correction), which appeared slightly more prevalent in populations with intermediate  
317 N/S morphologies (Supplementary Table S6). Allelic differentiation among the 26 samples was  
318 significant (Fisher's method;  $P<0.01$ ), allowing rejection of the null hypothesis that alleles are  
319 drawn from the same distribution in all samples. All between-site pairwise  $F_{ST}$ 's were also  
320 significant ( $P<0.01$  in all cases) which implied major genetic differentiation (results not shown).

321 Two genetically distinct clusters were detected by STRUCTURE/STRUCTURE  
322 HARVESTER (highest value of  $\Delta K = 52.67$ ) (Figure 5A,B). Although the approach used cannot  
323 reject one genetic cluster ( $K=1$ ), the significant genetic differentiation and clear geographical

324 structuring of the two genetic clusters rule this out: clusters were closely associated with the N/S  
325 variation in morphology. Also, sites containing individuals with Q values around 0.2-0.8  
326 (indicative of hybridization between individuals from different clusters) were most prevalent in  
327 areas of greatest morphological transition (Figure 5C). For example, the highest proportions of  
328 hybrid individuals (>40%) were found at sites 6 and 19.

329

### 330 *Analysis of N-S migration*

331 Replicated IMA2 analyses that started from different positions converged on the same posterior.  
332 The value of t corresponding to the highest posterior density (HiPt) scaled in years, was 258.5 ka  
333 (95% HPD: 168.3-630.5 ka). Population migration (2NM) is estimated as effective number of  
334 migrating gene copies per generation and was found to be high from the N to the S morph (HiPt:  
335 3.54, 95% HPD: 1.03-8.79) and differed significantly from zero (LRT:  $2\Delta l=7.487$ ,  $P<0.01$ )  
336 (Figure 6). The posterior on 2NM for migration of gene copies from the S to N morph was lower  
337 (HiPt: 0.022, 95% HPD: 0.00-6.00) and did not significantly differ from zero (LRT:  $2\Delta l=0.012$ ,  
338  $P>0.05$ ).

339

### 340 *Species distribution modelling*

341 The contribution of the available predictor variables varied considerably (Supplementary Table  
342 S3), but potential vegetation (20-23%) was most influential, followed by precipitation  
343 seasonality (6-8%). We selected the 7 variables that had a permutation importance of >5 for  
344 either the northern and/or the southern morph for use in SDM modelling of both N and S  
345 morphs. Generally high correlations were found between all climate variables ( $r>0.95$ ) although  
346 temperature and precipitation seasonality showed generally low correlations ( $r<0.62$ ) and so we



347 used these to provide alternative analyses of uncorrelated variables. Using the six climatic  
348 variables of high permutation importance plus potential vegetation, the species distribution  
349 models showed a high discriminatory power between presences and background. The AUCs for  
350 the calibration data sets were 0.823 for the northern morph, and 0.855 for the southern one (i.e.,  
351 82.3 and 85.5% of the records were correctly predicted, respectively). Randomization tests  
352 revealed that the AUCs were significant for both the northern ( $P=0.008$ ) and the southern  
353 ( $P=0.024$ ) morphs. Results were similar when we used just the two uncorrelated climatic  
354 variables (plus potential vegetation) instead of the six climatic variables with highest permutation  
355 importance. The SDMs were spatially non-overlapping for the N and S morphs indicating  
356 distinct environmental requirements (Figure 7).

357         Comparison of climate niche overlap between N and S morphs revealed significant  
358 deviation from the null distribution, indicating non-equivalence of niches between N and S  
359 morphs. This finding appears to be robust as it was supported by the analysis of 6 climatic  
360 variables with highest permutation importance ( $D=0.522$ ,  $P=0.0198$ ) as well as the alternative  
361 analysis of just two uncorrelated climatic variables ( $D=0.439$ ,  $P=0.0198$ ).

362

### 363 **Discussion**

364 We find evidence that within-island incipient speciation in *C. sexlineatus* is associated with both  
365 current ecological conditions and historical divergence in allopatry. We corroborate initial  
366 findings that the latter may have been mediated by volcanic activity through the creation of two  
367 or more disjunct habitat refuges within the island (Pestano and Brown 1999). This was achieved  
368 using more informative mtDNA sequences which allowed the detection of four well-supported  
369 mtDNA lineages (as opposed to three weakly supported lineages in Pestano and Brown [1999]).

370 We also analysed nuclear markers for the first time. The within-island morphological variation  
371 appears to correspond more closely to geographical structuring of nuclear microsatellite  
372 polymorphisms than to mtDNA phylogeography. This is not too surprising given that  
373 morphological differences should originate from divergence in the nuclear genome. Greatest  
374 levels of admixture are found in areas of intermediate morphology, as would be expected in a  
375 hybrid zone.

376 Environment-based distribution models of N and S morphs indicate close correspondence  
377 to the respective ecologically distinct N and S regions. This strengthens previous inferences that  
378 the morphs represent ecological forms that are adapted to the xeric and mesic habitat types  
379 (Brown et al. 1991). The isolation-with-migration analysis indicates that nuclear/mitochondrial  
380 gene flow is asymmetric with relatively high migration of gene copies from the N to the S morph  
381 but lower migration in the opposite direction. The N to S morph population migration estimate  
382 (3.5 migrant gene copies per generation) is much higher than the 0-1 range at which divergence  
383 is impeded (Slatkin 1995). Hence, strong habitat-related selection in the face of relatively high  
384 gene flow could explain the origin and maintenance of the southern morph, as expected under  
385 ecological divergence. The hypothesis that the southern morph has been subject to strong  
386 directional selection is further supported by the observation that it has quite a divergent  
387 morphology compared with the other Canary Island skinks of the same clade, at least in terms of  
388 colour which is indicative of different chromatophores in the skin (Kuriyama et al. 2006;  
389 Carranza et al. 2008). In contrast, the influx of southern gene copies into the northern morph  
390 appears negligible, possibly explaining why it remains morphologically distinct from the  
391 southern morph. An inability to survive and reproduce in foreign habitats has been described in  
392 several taxa (see Nosil et al. 2005) and could potentially explain this restricted introgression. The

393 present data do not reveal why gene flow appears to be asymmetric.

394         How the two morphs may have evolved under different selection pressures has been  
395 discussed previously (Brown and Thorpe 1991b). It was postulated that uniform brown northern  
396 skinks were suited to the more mesic N areas because they allow a more cryptic anti-predation  
397 strategy against birds such as the kestrel. The bright blue-tailed skinks appear suited to the more  
398 open xeric S areas where crypsis may be less successful. Escape from anti-predator attacks in  
399 these open habitats would be achieved by attracting predatory attacks towards the tail, which can  
400 autotomize increasing the chances of escape. It has been observed that lizards with distinctive  
401 tail colorations tend to be associated with more open habitats (Arnold 1984) which fits well with  
402 the pattern on Gran Canaria. The finding of a parallel, albeit weaker, pattern of tail colour  
403 variation in *Chalcides* from the neighbouring island of Tenerife, also supports this hypothesis  
404 (Brown et al. 1991).

405         Despite support for ecological divergence there is also clear evidence of additional  
406 historical vicariance. Population divergence began during the early Pleistocene or the late  
407 Pliocene and is one element that supports the role of the last major eruptive cycle in Gran  
408 Canaria, 1.5-3 Ma ago. During this period, eruptions covered most of the NE of the island with  
409 lava to approximately 500m depth, except for an isolated area in the extreme NE close to the  
410 current location of the city of Las Palmas (Carracedo 2011, and references therein) providing an  
411 isolated northern refuge. The south of the island was largely unaffected by these eruptions.  
412 Hence the spatial correspondence between these eruptions and the observed N/S genetic pattern  
413 adds support to the volcanism-mediated geographical isolation hypothesis. Finally, the finding of  
414 a strong signal of population increase found in the N mtDNA is concordant with range expansion  
415 from a northern volcanic refuge. The earliest split between the W, SE and N mtDNA lineages

416 (1.5 Ma ago) also fits in with the timing of northern volcanic activity and may have been part of  
417 this process, although the most recent split between these clades (0.4 Ma ago) is clearly too early  
418 to be associated with this period.

419 It is worth considering why the phylogenetic multispecies coalescent analysis provided a  
420 very different estimate of N-S divergence time (2 Ma) to the analysis of isolation-with-migration  
421 (260 ka) with non-overlapping posterior intervals. The isolation-with-migration analysis depends  
422 on an estimate of generation time (and of mutation rate but this was derived from the \*BEAST  
423 analysis) but even an error as large as 50% in this estimate would not explain the difference.  
424 Instead, it is more likely to be because our \*BEAST analyses simply date the divergence  
425 between distinct mtDNA lineages, equivalent to an analysis on two completely sorted  
426 populations. This was a suitable approach given that multispecies coalescent analyses do not take  
427 gene flow into account. In contrast, the IMA2 analysis examines both splitting time and  
428 microsatellite/mtDNA gene flow between the two morphological groups, which share  
429 microsatellite alleles and mtDNA haplotypes. Thus, the \*BEAST analyses should provide a  
430 better estimate of divergence time of mtDNA lineages, while IMA2 may confound divergence  
431 time with levels of gene flow. Nevertheless, if IMA2 incorrectly attributed greater similarity  
432 between populations to more recent divergence rather than high gene flow, then this would lead  
433 to migration of gene copies being underestimated which would not affect our inferences.

434 One final cautionary point about the IMA2 analyses is that we cannot establish the relative  
435 influences of mtDNA and microsatellites on the results. Test analyses on microsatellite loci alone  
436 did not provide reliable posterior distributions and therefore are not helpful. Clearly, migration of  
437 nuclear alleles should be more relevant to morphological divergence than mtDNA migration, but  
438 we cannot decisively show that a significant component of the observed migration is accounted

439 for by microsatellite alleles.

440 In summary, our analyses support the ecological origins of the two primary skink morphs  
441 because their current distributions can be largely predicted from bioclimatic modelling. The  
442 finding of high rates of migration of gene copies from N to S suggest that these differences are  
443 maintained by strong selection pressures, at least within the arid southern habitats. These effects  
444 seem to be additional to ancient population vicariance mediated by Pleistocene volcanic activity  
445 in NE Gran Canaria. Studies of population divergence frequently focus on one particular causal  
446 mechanism in isolation, but here we show how different processes can combine to shape genetic  
447 and morphological diversity within a very small geographic area.

448

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456

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587

### 588 **Data Accessibility**

- 589 - DNA sequences: GeneBank accession numbers: KJ463905-KJ464030
- 590 - Microsatellite genotypes: Dryad doi: <http://dx.doi.org/10.5061/dryad.db451/1>
- 591 - MtDNA alignment and partition data: Dryad doi: <http://dx.doi.org/10.5061/dryad.db451/2>
- 592 - Occurrence data: Dryad doi: <http://dx.doi.org/10.5061/dryad.db451/3>
- 593 - Species Distribution Modelling data: Dryad doi: <http://dx.doi.org/10.5061/dryad.db451/4>

594

### 595 **Author Contributions**

596 This work originated from NMS's PhD that he carried out in JPs laboratory at the University of  
597 Las Palmas. The study was originally formulated by RPB during an EU research fellowship held  
598 at the University of Las Palmas. NMS and RPB recently reanalysed the data and wrote the paper.

599

600 **Supporting Information**

601 Figures S1

602 Tables S1-6

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603 **Figure Legends**

604 Figure 1. Geographical locations of *C. sexlineatus* sample sites. The line across the island  
605 represents the midpoint of the N/S morphological variation (Brown & Thorpe 1991b).

606

607 Figure 2. The 50% majority rule consensus of the posterior mtDNA trees obtained from the  
608 Bayesian analysis. Bayesian posterior probabilities are shown at each node. The geographical  
609 distributions of the four main lineages are shown on the map, as well as the areas affected by  
610 volcanism (dark shading: Holocene volcanism, medium shading: rift volcanism 1.5-3 Ma, light  
611 shading: inferred rift volcanism with the rift axis shown as a dotted line (adapted from  
612 (Carracedo 2011).

613

614 Figure 3. Bayesian skyline plots showing estimated demographic changes over time in the four  
615 mtDNA lineages. Lines represent posterior medians (continuous), upper and lower 95% HPDs  
616 (dotted).

617

618 Figure 4. \*BEAST population tree chronogram. Median posterior ages of nodes are provided,  
619 together with bars representing 95% HPDs. Scale bar provides times in millions of years.

620

621 Figure 5. Genetic structure inferred from microsatellites using STRUCTURE. A) Individual  
622 assignment to clusters ( $K=2$ ) based on B)  $\Delta K$  (Evanno *et al.* 2005). C) Site compositions.

623

624 Figure 6. Posterior densities for population migration (2NM) estimated using IMa2.

625

626 Figure 7. Species distribution models for the northern and southern morphs. Higher values  
627 indicate higher predicted environmental suitability.

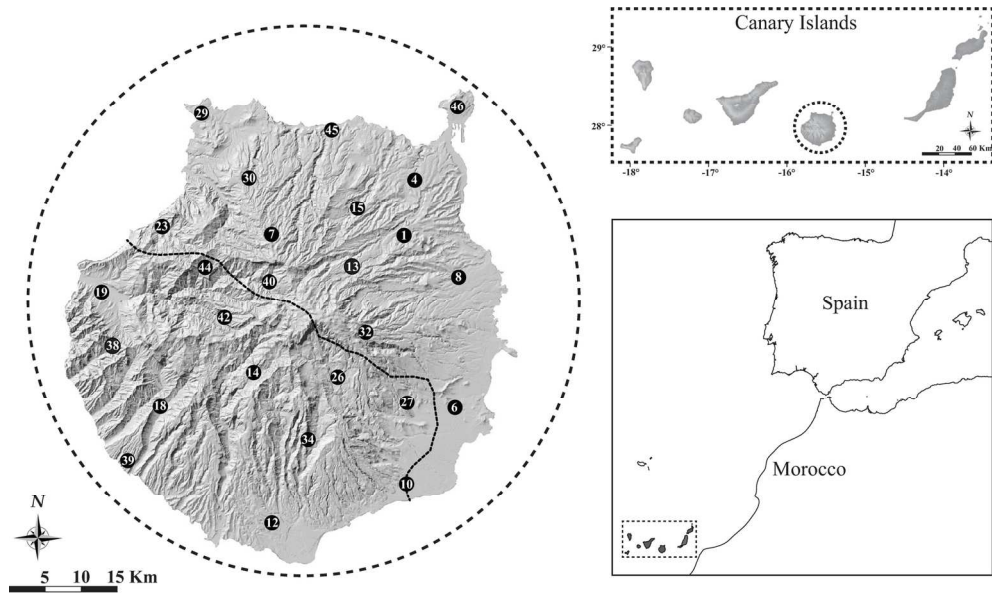
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Table 1. Summary statistics for the four main mtDNA lineages identified in *C. sexlineatus*: *n*, number of individuals; PS, number of polymorphic sites; NH, number of haplotypes;  $R^2$ , Ramos-Onsins and Rozas statistic (Ramos-Onsins & Rozas 2002). \* $P < 0.1$ , \*\* $P < 0.05$ , \*\*\* $P < 0.001$ .

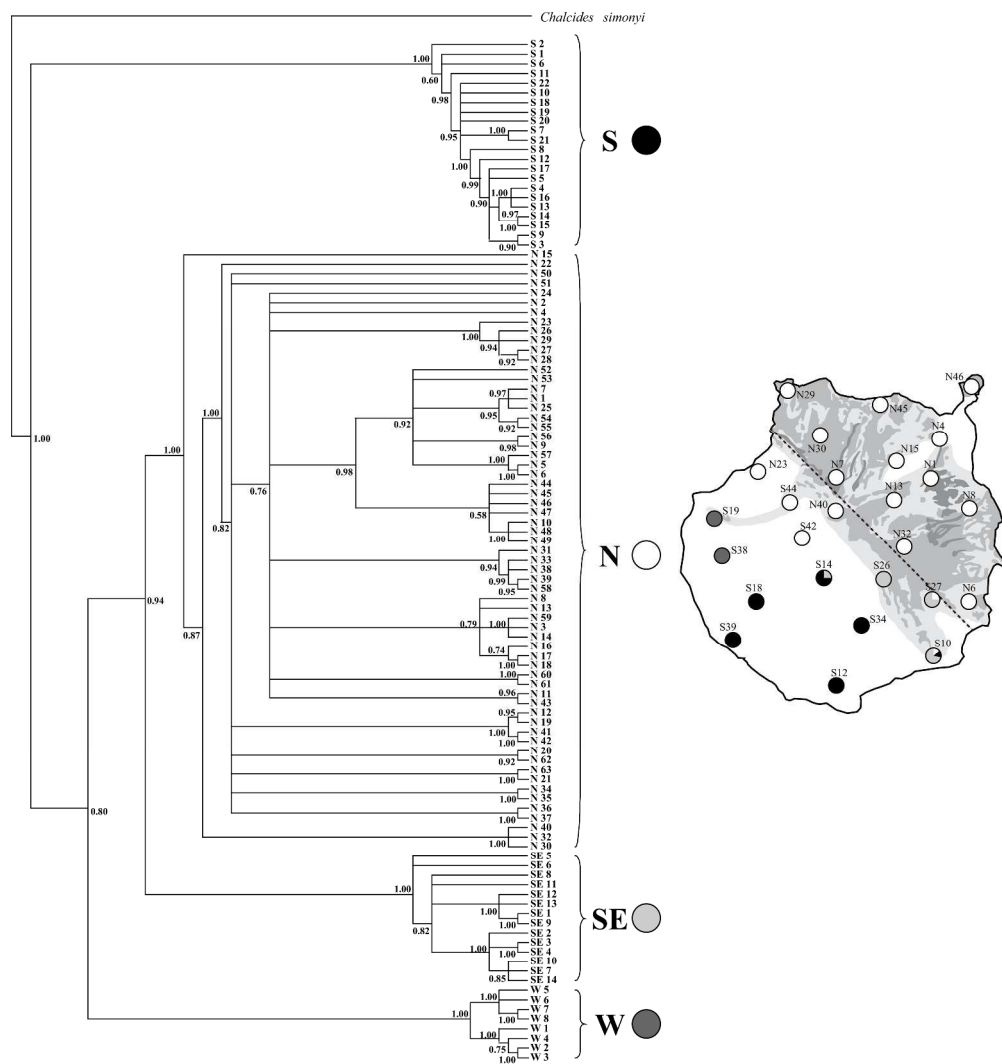
Lineage	<i>n</i>	PS	Parsimony informative sites	NH	Haplotype diversity	Nucleotide diversity	$R^2$	Fu's $F_s$ (1997)	Fu & Li's D (1993)	Fu & Li's F (1993)	Tajima's D (1989)
North	78	139	89	63	0.994	0.012	0.0401**	-48.11***	-1.820 <sup>ns</sup>	-2.280 <sup>ns</sup>	-2.016**
South	23	70	28	22	0.996	0.012	0.0627**	-10.90***	-2.112 <sup>ns</sup>	-2.282 <sup>ns</sup>	-1.580 <sup>ns</sup>
South-East	19	35	19	14	0.953	0.007	0.0906*	-3.38*	-0.858 <sup>ns</sup>	-1.043 <sup>ns</sup>	-0.992 <sup>ns</sup>
West	17	51	36	8	0.838	0.017	0.1585 <sup>ns</sup>	5.16 <sup>ns</sup>	0.035 <sup>ns</sup>	0.218 <sup>ns</sup>	0.566 <sup>ns</sup>
All	137	234	183	107	0.995	0.036	0.0743 <sup>ns</sup>	-46.57***	-0.990 <sup>ns</sup>	-1.088 <sup>ns</sup>	-0.813 <sup>ns</sup>



Geographical locations of *C. sexlineatus* sample sites. The line across the island represents the midpoint of the N/S morphological variation (Brown & Thorpe 1991b).  
156x91mm (300 x 300 DPI)

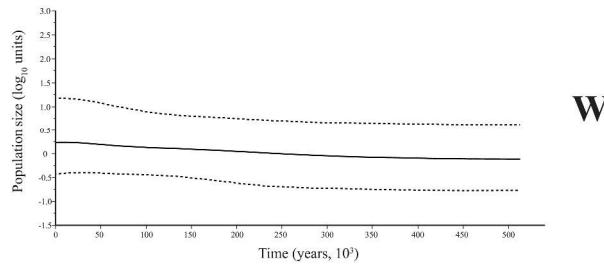
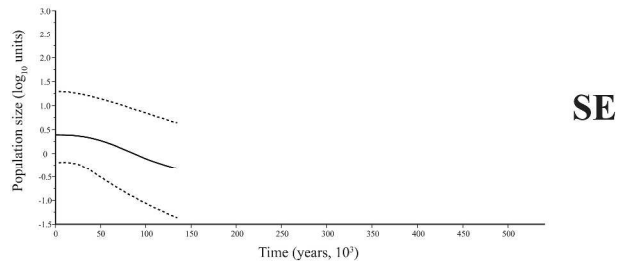
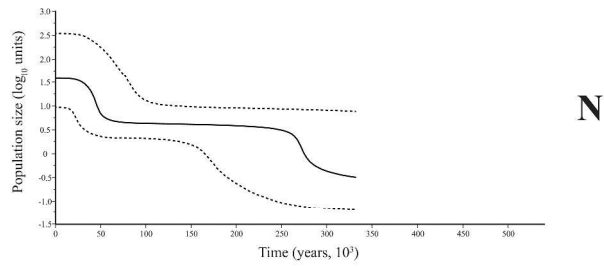
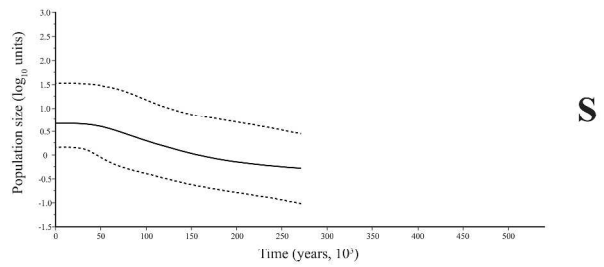
View Only



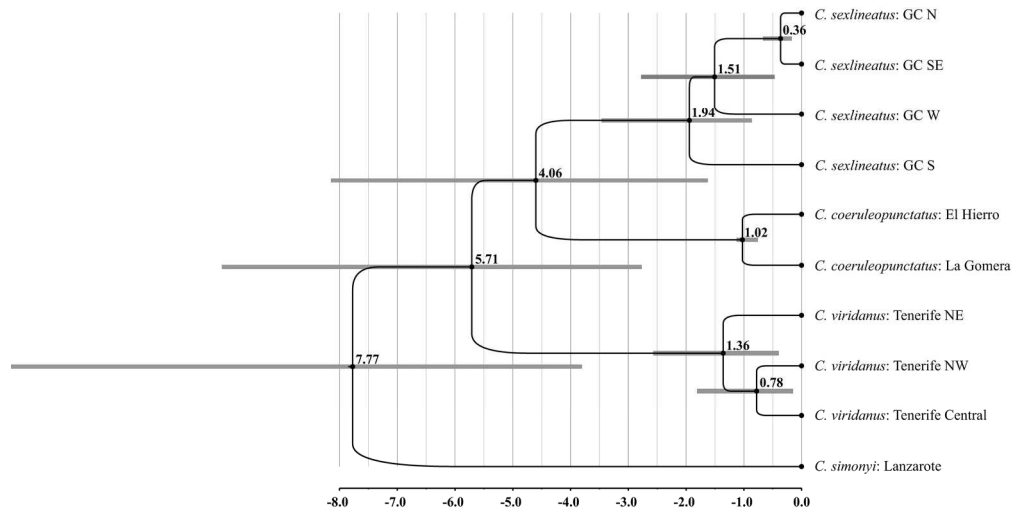


The 50% majority rule consensus of the posterior mtDNA trees obtained from the Bayesian analysis. Bayesian posterior probabilities are shown at each node. The geographical distributions of the four main lineages are shown on the map, as well as the areas affected by volcanism (dark shading: Holocene volcanism, medium shading: rift volcanism 1.5-3 Ma, light shading: inferred rift volcanism with the rift axis shown as a dotted line (adapted from (Carracedo 2011).

346x365mm (300 x 300 DPI)

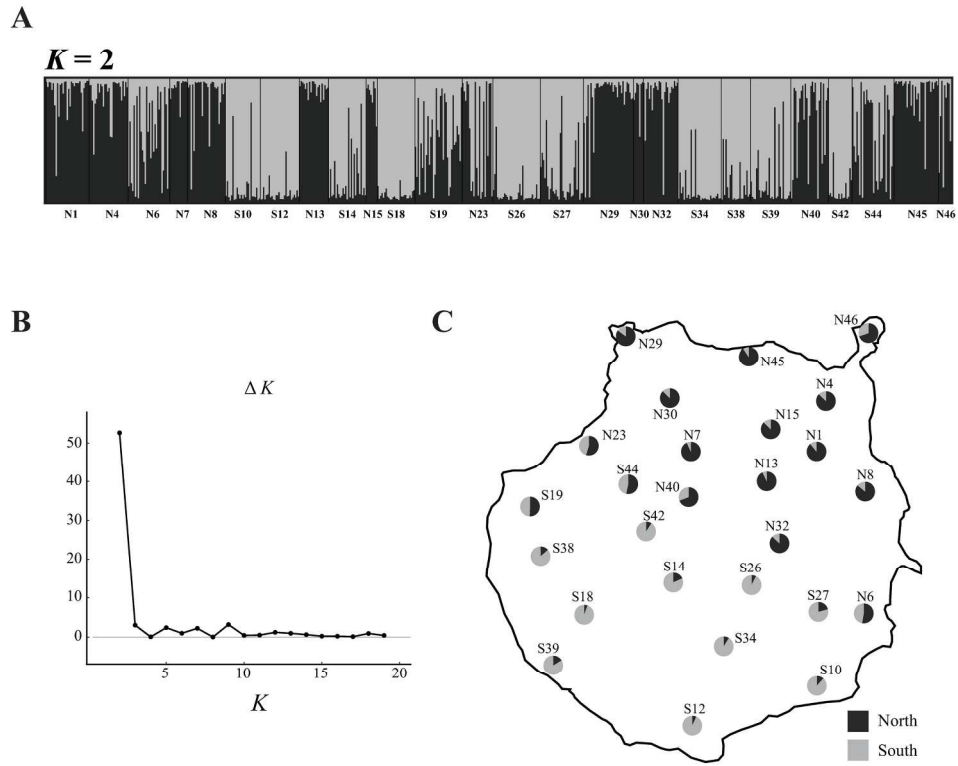


Bayesian skyline plots showing estimated demographic changes over time in the four mtDNA lineages. Lines represent posterior medians (continuous), upper and lower 95% HPDs (dotted).  
449x695mm (600 x 600 DPI)



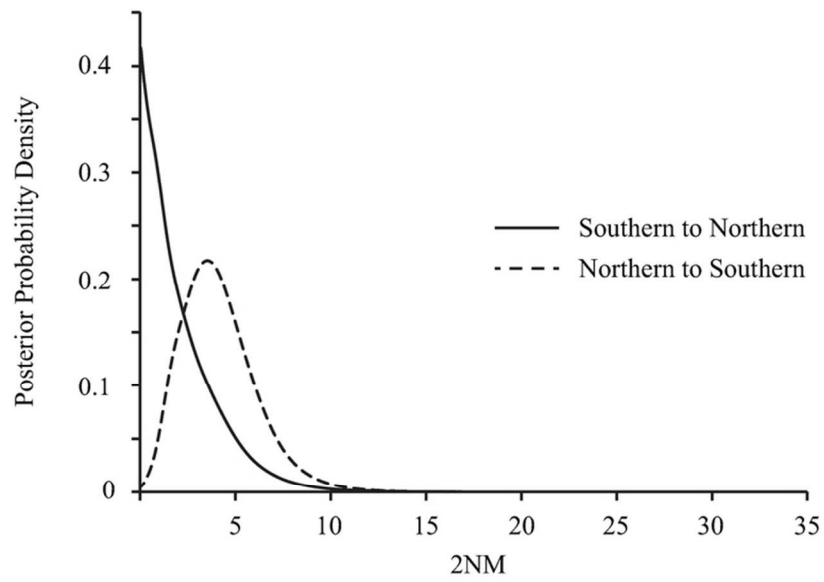
\*BEAST population tree chronogram. Median posterior ages of nodes are provided, together with bars representing 95% HPDs. Scale bar provides times in millions of years.  
184x92mm (300 x 300 DPI)

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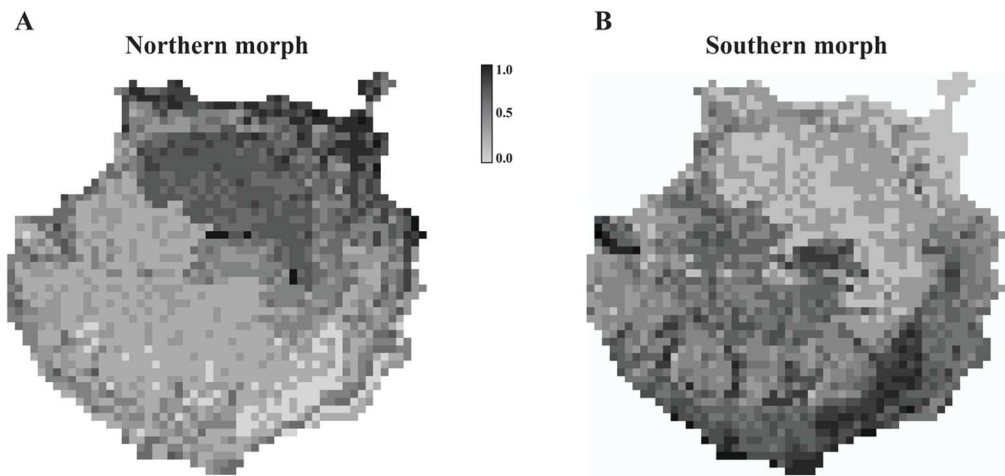
Genetic structure inferred from microsatellites using STRUCTURE. A) Individual assignment to clusters ( $K=2$ ) based on B)  $\Delta K$  (Evanno et al. 2005). C) Site compositions.  
206x170mm (300 x 300 DPI)

Only



Posterior densities for population migration (2NM) estimated using IMA2.  
79x48mm (300 x 300 DPI)

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Species distribution models for the northern and southern morphs. Higher values indicate higher predicted environmental suitability.  
122x57mm (300 x 300 DPI)

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