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MOLECULAR ECOLOGY**Ecological divergence combined with ancient allopatry in lizard populations from a small volcanic island**

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1 **Ecological divergence combined with ancient allopatry in lizard populations from a small**
2 **volcanic island**

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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 km² 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^{Ile}, tRNA^{Gln} and tRNA^{Met}). Polymerase chain reactions
118 (PCRs) were carried out in 25 µl volumes using: 20–50 ng DNA, 1X buffer (Bioline; 16 mM
119 (NH₄)₂SO₄, 67 mM Tris–HCl (pH 8.8) and 0.01% Tween 20), 1.5 mM MgCl₂, 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[®] PCR System 2700 (Applied Biosystems). PCR products were
126 purified using MicroSpin[™] 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_s [(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) 1st codon positions (ND1+ND2), 2) 2nd codon
137 positions (ND1+ND2), 3) 3rd 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 2×10^6 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). 4×10^5 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 4×10^7 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 ΔK 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 1.01×10^8 steps, with parameters
236 sampled every 100 steps, and the first 1×10^6 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 1.8351×10^{-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 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 475th 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^{Ile}, 77-79 bp; tRNA^{Gln}, 71 bp; tRNA^{Met}, 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

For Review Only

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) ΔK (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.

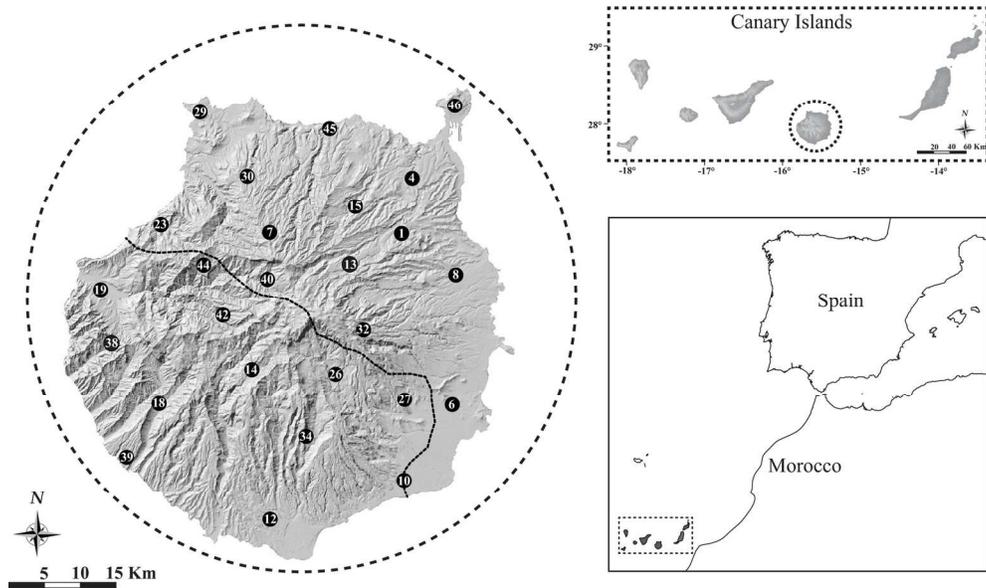
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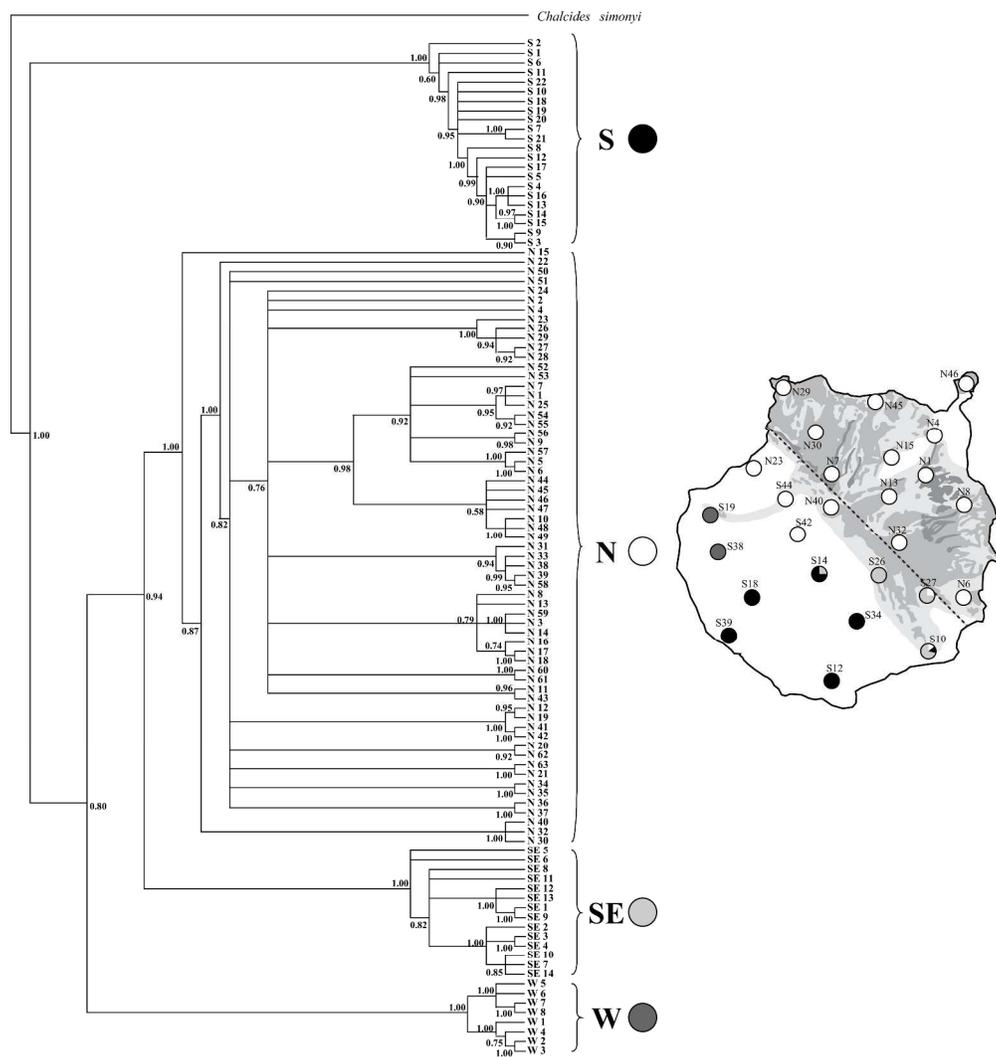
Table 1. Summary statistics for the four main mtDNA lineages identified in *C. sexlineatus*: *n*, number of individuals; PS, number of polymorphic sites; NH, number of haplotypes; R^2 , Ramos-Onsins and Rozas statistic (Ramos-Onsins & Rozas 2002). * $P < 0.1$, ** $P < 0.05$, *** $P < 0.001$.

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 ^{ns}	-2.280 ^{ns}	-2.016**
South	23	70	28	22	0.996	0.012	0.0627**	-10.90***	-2.112 ^{ns}	-2.282 ^{ns}	-1.580 ^{ns}
South-East	19	35	19	14	0.953	0.007	0.0906*	-3.38*	-0.858 ^{ns}	-1.043 ^{ns}	-0.992 ^{ns}
West	17	51	36	8	0.838	0.017	0.1585 ^{ns}	5.16 ^{ns}	0.035 ^{ns}	0.218 ^{ns}	0.566 ^{ns}
All	137	234	183	107	0.995	0.036	0.0743 ^{ns}	-46.57***	-0.990 ^{ns}	-1.088 ^{ns}	-0.813 ^{ns}



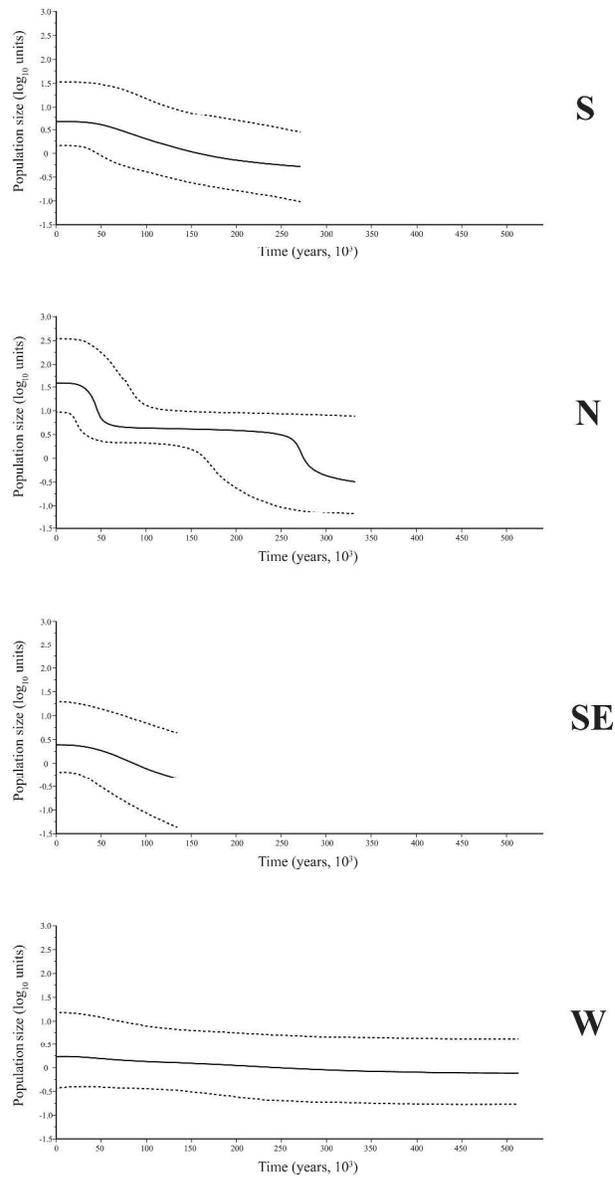
Geographical locations of *C. sexlineatus* sample sites. The line across the island represents the midpoint of the N/S morphological variation (Brown & Thorpe 1991b).
156x91mm (300 x 300 DPI)

View Only

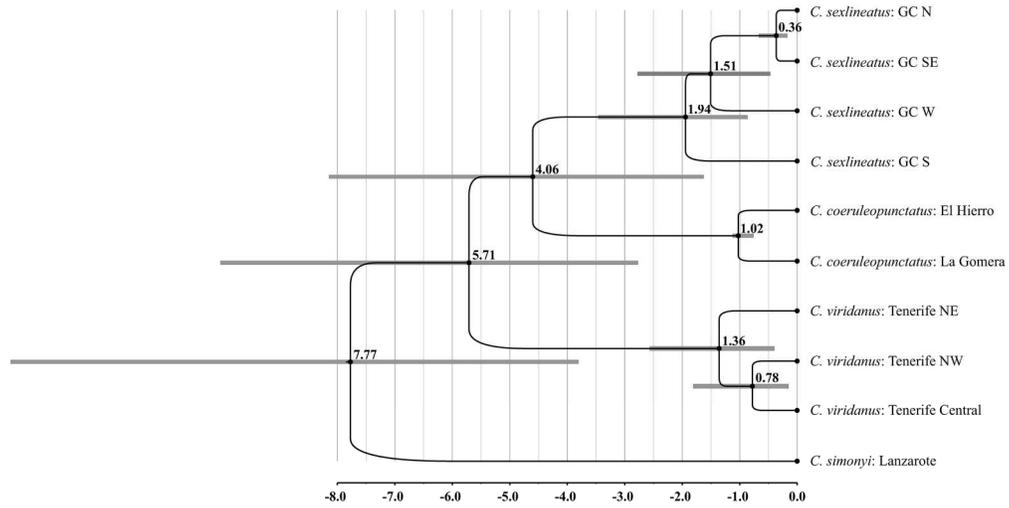


The 50% majority rule consensus of the posterior mtDNA trees obtained from the Bayesian analysis. Bayesian posterior probabilities are shown at each node. The geographical distributions of the four main lineages are shown on the map, as well as the areas affected by volcanism (dark shading: Holocene 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)

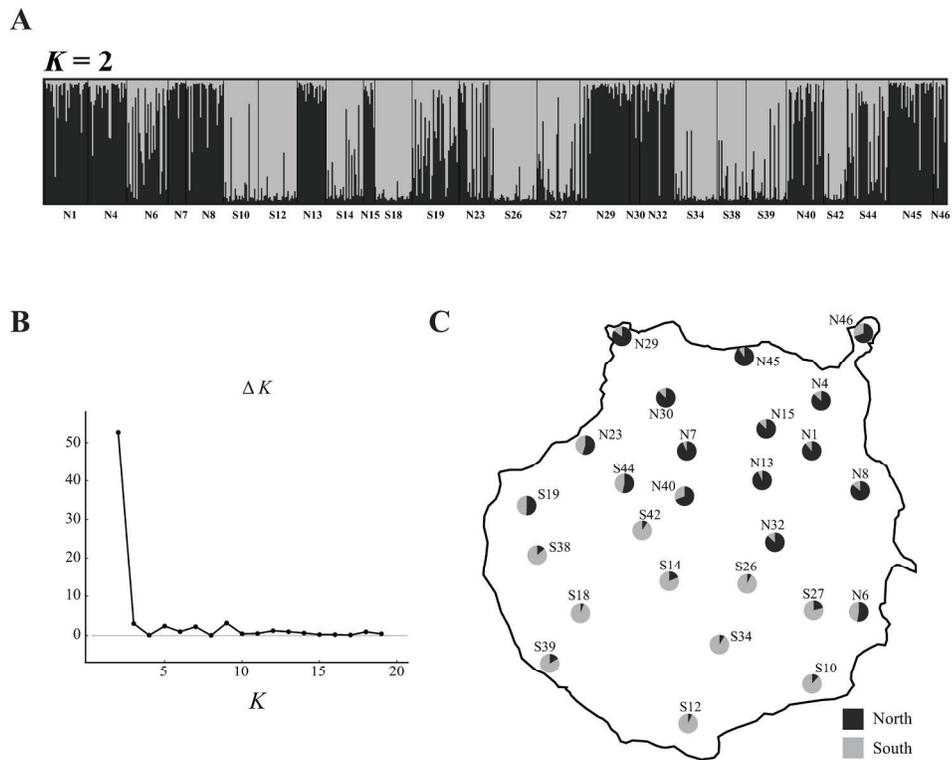


Bayesian skyline plots showing estimated demographic changes over time in the four mtDNA lineages. Lines represent posterior medians (continuous), upper and lower 95% HPDs (dotted).
449x695mm (600 x 600 DPI)

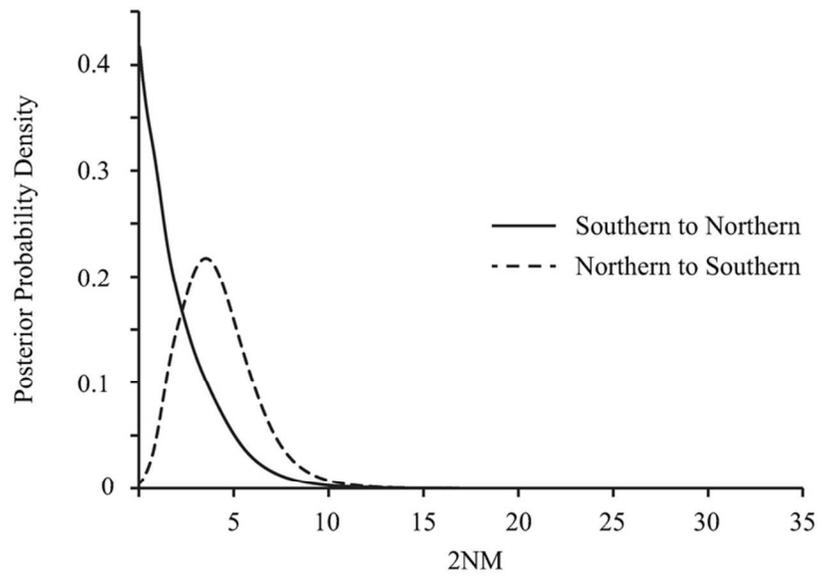


*BEAST population tree chronogram. Median posterior ages of nodes are provided, together with bars representing 95% HPDs. Scale bar provides times in millions of years.
184x92mm (300 x 300 DPI)

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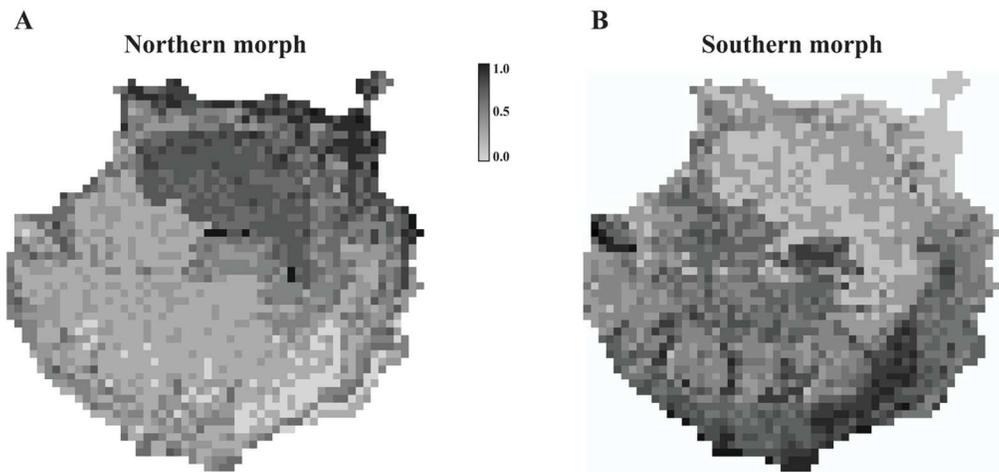


Genetic structure inferred from microsatellites using STRUCTURE. A) Individual assignment to clusters ($K=2$) based on B) ΔK (Evanno et al. 2005). C) Site compositions.
206x170mm (300 x 300 DPI)



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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