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1 Chloroplast microsatellites reveal colonisation and metapopulation dynamics in the
2 Canary Island pine

3

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15

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18

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28

29 **ABSTRACT**

30 Chloroplast microsatellites are becoming increasingly popular markers for population
31 genetic studies in plants, but there has been little focus on their potential for
32 demographic inference. In this work the utility of chloroplast microsatellites for the
33 study of population expansions was explored. First, we investigated the power of
34 mismatch distribution analysis and the F_S test with coalescent simulations of different
35 demographic scenarios. We then applied those methods to empirical data obtained for
36 the Canary Island pine (*Pinus canariensis*). The results of the simulations showed that
37 chloroplast microsatellites are sensitive to sudden population growth. The power of
38 the F_S test and accuracy of demographic parameter estimates, such as the time of
39 expansion, were reduced proportionally to the level of homoplasy within the data. The
40 analysis of Canary Island pine chloroplast microsatellite data indicated population
41 expansions for almost all sample localities. Demographic expansions at the island
42 level can be explained by the colonisation of the archipelago by the pine, while
43 population expansions of different ages in different localities within an island appear
44 to be the result of local extinctions and recolonisation dynamics. Comparable
45 mitochondrial DNA sequence data from a parasite of *P. canariensis*, the weevil
46 *Brachyderes rugatus*, supports this scenario, suggesting a key role for volcanism in
47 the evolution of pine forest communities in the Canary Islands.
48

49 **INTRODUCTION**

50

51 In plants the chloroplast genome is used extensively for evolutionary genetic studies
52 within species in the same way the mitochondrial genome is used within animal
53 studies. However, finding enough sequence variation is a challenge due to the low
54 mutation rates that characterize the chloroplast genome. In contrast, chloroplast
55 microsatellites, or simple sequence repeats (cpSSRs), present higher levels of
56 polymorphism and are easily genotyped and this has made them useful and popular
57 markers for population genetic studies (Provan *et al.*, 2001). Although used
58 extensively for studying population structure and gene flow, the potential of cpSSRs
59 to study population demographic history has received little attention. In this study we
60 investigate in the utility of cpSSR data for the detection of population expansions.

61

62 The study of historical demography by means of genetic information is based on
63 coalescent theory (see Emerson *et al.*, 2001 for a review). In a stable population
64 coalescence events are scarcer towards the past giving a genealogy dominated by an
65 ancient bifurcation with mutations mainly distributed in inter-node branches (King *et al.*,
66 2000; Reich & Goldstein, 1998). Contrastingly, in the case of sudden population
67 growth, coalescent events occur mainly during the expansion, leaving a “comblike”
68 genealogy; and mutations are more abundant along the terminal branches (singleton
69 mutations) than in inter-node branches (figure 1 shows the main differences of the two
70 opposing scenarios). As a consequence, population expansions can be detected
71 because of an excess of singletons (Fu & Li, 1993; Tajima, 1989) or an excess of
72 haplotypes (as a consequence of the excess of singletons, Fu, 1997). Also, the
73 divergence between most lineages dates from the time of expansion, producing
74 unimodal distributions of pairwise genetic distances (Slatkin & Hudson, 1991). The
75 study of such distributions also allows for the estimation of the time and magnitude of
76 the population increase (Rogers, 1995; Schneider & Excoffier, 1999).

77

78 The methods for studying population expansions are fairly robust for a genetic marker
79 evolving under the unrealistic infinite sites model, where singletons and genetic
80 distances are identified without error. However, in the evolution of sequences under a
81 finite sites model, parallel and back mutations (i.e. homoplastic mutations) will erase
82 part of the genetic information producing inaccurate estimates of singletons and
83 genetic distances. This affects the power of the statistical tests and the estimates of
84 time and magnitude of the demographic growth (Aris-Brosou & Excoffier, 1996;
85 Bertorelle & Slatkin, 1995). In nucleotide sequence data, the usual markers for
86 studying population expansions, the effect of homoplasy is small (Rogers *et al.*, 1996)
87 and can be accounted for in more sophisticated analyses (Schneider & Excoffier,
88 1999). In cpSSRs, which evolve in a stepwise fashion, higher levels of homoplasy are
89 expected in comparison with sequence data and therefore statistical analyses
90 developed for DNA sequence data may prove unreliable.

91

92 In the present work we have simulated the evolution of cpSSRs under constant
93 population size and under population expansion to test the usefulness of these markers
94 for the study of demographic expansions. These theoretical results were then
95 compared with empirical results from the Canary Island pine (*Pinus canariensis*). The
96 presence of *P. canariensis* on each of the five volcanic islands on which it occurs
97 must be through colonisation after the emergence of each island, followed by
98 population expansion.

99

100 MATERIALS AND METHODS

101

102 *Simulations*

103

104 Demographic histories of population expansions (recent and old) and stable
105 population size were modelled with coalescent simulations to obtain theoretical
106 expectations of the behaviour of cpSSRs. The coalescent simulation (described in
107 Navascués & Emerson, 2005) consists of the generation of a genealogy for a sample
108 of individuals under a particular demographic history followed by the distribution of
109 mutations randomly onto those lineages. For the population expansions the
110 demographic history was modelled with a logistic equation setting the initial
111 population size (N_0) as one individual (coloniser) at the time of expansion (τ , in
112 mutational units). Microsatellite evolution was simulated following a symmetrical
113 single-step mutation model where mutation rates were either heterogeneous (two-rates
114 model) or uniform (one-rate model) across loci. Heterogeneous mutation rates can be
115 considered a more realistic scenario taking into account the differences in
116 polymorphism among cpSSR loci (see, for example, Gómez *et al.*, 2003). As well as
117 being more realistic, heterogeneous mutation rates will also produce higher levels of
118 homoplasy by concentrating the mutations onto particular loci, thus providing a more
119 rigorous assessment of the demographic utility of cpSSRs. The three different
120 demographic histories and the two mutation models gave a combination of six
121 different cases considered (table 1). Simulations were performed for a sample size of
122 24 individuals and six cpSSR loci. For each case, 1000 replicates were run and their
123 output (genetic state of a sample of individuals in the present generation) was
124 analysed as described in section (c). For each simulated case the level of homoplasy
125 was quantified as the probability that two haplotypes identical in state are not identical
126 by descent (homoplasy index, Estoup *et al.*, 2002).

127

128 *Plant Material and Molecular Markers*

129

130 Empirical data was obtained from two previous studies of *P. canariensis* (Gómez *et al.*
131 *et al.*, 2003; Vaxevanidou *et al.*, 2005). Additionally, three populations from Tenerife
132 (nine, 12 and 13 in table 2 and figure 2) were also genotyped for the present analysis
133 and the compatibility of the data was assured by repeated genotyping of four
134 haplotypes from the previous studies. All individuals were genotyped for six cpSSR
135 loci: Pt15169, Pt30204, Pt71936, Pt87268, Pt26081 and Pt36480 (Vendramin *et al.*,
136 1996).

137

138 *Data Analysis*

139

140 In order to use Arlequin 2.0 (Schneider *et al.*, 1999) for the analyses, microsatellite
141 data was binary coded: the number of repeats were coded with “1” and shorter alleles
142 were coded filling the difference in repeats with “0” (Pereira *et al.*, 2002). Analyses
143 for the empirical samples were carried out at two levels: (1) sample sites as the unit of
144 analysis, (2) islands as the unit of analysis with sample sites within an island pooled
145 together.

146

147 A general description of diversity indices and population structure found within *P.*
148 *canariensis* using cpSSRs is presented in Gómez *et al.* (2003); thus here we focus on

149 the assessment of demographic history, using two different but complementary
150 approaches. Firstly, we performed the F_S neutrality test for population expansion (Fu,
151 1997). This test is based on different expectations for the number of haplotypes when
152 comparing a stationary with an expansion demography. The F_S statistic takes a large
153 negative value within a population affected by expansion due to an excess of rare
154 haplotypes (recent mutations). Significance of the test was calculated with 10 000 data
155 bootstraps (Schneider *et al.*, 1999). An F_S statistic with $p(F_S) < 0.02$ ($\alpha = 0.05$, due to
156 a particular behaviour of this statistic, Fu, 1997) was considered evidence of
157 population expansion.

158
159 The second analysis consists of the estimation of the demographic model of Rogers &
160 Harpending (1992) described with the parameters: $\tau = 2\mu t$, $\theta_0 = 2\mu N_0$ and $\theta_1 = 2\mu N_1$
161 (where μ is the mutation rate, t is the number of generations since expansion and N_0
162 and N_1 are the population sizes before and after expansion). Parameters are estimated
163 from the distribution of pairwise differences (difference in number of repeats)
164 between individuals within a sample. Although, in our case, the pairwise differences
165 calculated cannot be strictly called mismatches, we will refer to their distribution as a
166 mismatch distribution as it is the most common term used throughout the literature
167 (Harpending *et al.*, 1993). This distribution is affected by the demography of the
168 sample; sudden growth produces unimodal distributions while within stationary
169 populations distributions are ragged and multimodal (Slatkin & Hudson, 1991). An
170 algorithm, which minimizes the sum of squared differences (SSD) between model and
171 data, estimates the combination of parameters with the best fit to the empirical data
172 (Schneider & Excoffier, 1999). The strength of the estimated model is then evaluated
173 from the SSD distribution which is obtained from 10 000 data bootstraps (1000 for the
174 simulation output), making $p(\text{SSD})$ the proportion of bootstraps with the SSD larger
175 than the original (Schneider & Excoffier, 1999). A significant SSD value, $p(\text{SSD}) <$
176 0.05 , implies the rejection of the estimated demographic model. The confidence
177 interval (95% CI) for the estimated parameter $\hat{\tau}$ is also calculated from the bootstrap
178 process (Schneider & Excoffier, 1999). Confidence intervals for parameters related to
179 the magnitude of expansion ($\hat{\theta}_0$ and $\hat{\theta}_1$) will not be discussed as they are usually too
180 wide and are of less interest for the interpretation of the results (Excoffier &
181 Schneider, 1999). Dating the population expansions was done using the parameter $\hat{\tau}$
182 and its relationship with time and mutation rate: $\tau = 2l\mu t$ (where l is the number of
183 cpSSR loci and μ is the mutation rate per locus).

184 185 **RESULTS AND DISCUSSION**

186 187 ***Simulations***

188 The results from the simulations are summarised in table 1. In the two analyses
189 performed, cpSSR polymorphism was sensitive to population growth; however the
190 results were not as precise as would be desirable.

191 192 ***F_S Neutrality Test***

193 In the cases of uniform mutation rate across loci the performance of the F_S test to
194 detect population expansion was acceptable. Type II error for the F_S test (no evidence
195 of population expansion in cases 1 and 2) was very low, and type I error (rejection of
196 stationary population size in case 3) was low (11% of the replicates of case 3),
197 although greater than expected at the given confidence level (expected 5% for
198 $\alpha=0.05$).

199

200 In the cases evolving under the two-rate model (cases 4–6), the power of the F_S test
201 decreased dramatically, and this was accompanied by an increase in homoplasy.
202 Detection of recent expansions was especially affected and the reason for this relates
203 to the estimates of genetic distance and the number of haplotypes used in the test.
204 First, the test uses the average genetic distance among individuals to calculate the
205 expected number of haplotypes under a stationary demography scenario. The effect of
206 homoplasy in this calculation is proportional to the time of expansion, with an average
207 reduction of 19% in the distance estimates of recent expansions (case 4) and 41% in
208 the older expansions (case 5). The expected number of haplotypes is then compared to
209 the observed number of haplotypes. While the effect of homoplasy in genetic distance
210 estimates was proportional to the time of expansion, homoplasy decreases the
211 detectable number of haplotypes by approximately 40% both in the recent and older
212 expansions. It seems that the power of the test varies with the time of expansions
213 because the error in the estimates of genetic distances and number of haplotypes is
214 more unbalanced for recent expansions.

215

216 *Demographic Model Estimation*

217 For cpSSRs evolving under the one-rate mutation model, estimates of the time of
218 expansion were fairly accurate, although older expansion times were slightly
219 underestimated. The average estimated time of expansion ($\hat{\tau}$) for the recent
220 expansions (case 1, $\tau = 1.0$) was 1.1 and the true value was always within the 95% CI,
221 while for older expansions (case 2, $\tau = 3.0$) average $\hat{\tau}$ was 2.5 and the true value falls
222 outside the 95% CI in 15% of the replicates. In the simulations using the two-rate
223 mutation model the estimates for recent expansion (case 5, $\tau = 1.0$) were accurate,
224 with average $\hat{\tau} = 1.0$ and the true value fell outside the 95% CI in only 2% of the
225 replicates. However in older expansions (case 6, $\tau = 3.0$) the expansion time was
226 largely underestimated for the two-rate mutation model with the average value of $\hat{\tau}$
227 being 1.8 and the true value falling outside the 95% CI in 77% of the replicates.
228 Although these results appear discouraging it is important to note that the relative
229 times of expansion are still discernable, and that it may be possible to develop new
230 statistical analyses to improve the estimates as has been done for heterogeneous
231 mutation rates within sequence data (Schneider & Excoffier, 1999).

232

233 *The Empirical Case: Pinus canariensis*

234 The results for the detection of population expansions in the *P. canariensis* samples
235 are reported in table 2. For the estimation of the demographic model the algorithm
236 was unable to find a combination of parameters with a minimum SSD in three
237 samples (Tamadaba, Chinyero and El Hierro). This inability of the algorithm to
238 converge sometimes has been observed in previous works (e.g. Stamatis *et al.*, 2004)
239 and in our simulations. A simple solution is to obtain the estimation from a reduced
240 sample obtained by randomly removing one individual. This reduction of the sample
241 size changes the shape of the mismatch distribution slightly enough for the algorithm
242 to converge while still maintaining a very similar shape to the mismatch distribution
243 from the original data set. The mismatch distributions from the reduced samples were
244 used to produce the parameter estimations presented in italics in table 2.

245

246 The demographic expansion model estimated for different sampling sites (including
247 the grouping of sampling sites at the island level) was, in general, fairly robust
248 [$p(\text{SSD}) \gg 0.05$] and mismatch distributions were clearly unimodal (figure 2;

249 opposite to the ragged distribution expected with a stable population). The results of
250 the F_S test yielded evidence of population expansion for nearly half of the samples. It
251 is interesting to note that the samples for which the F_S test could not reject a stable
252 population scenario [$p(F_S) > 0.02$] were the ones with the lowest $\hat{\tau}$ values. In the light
253 of our simulation results it is expected that the F_S test will have lower power to detect
254 very recent population expansions, especially under the more realistic scenario of
255 heterogeneous mutation rates across loci. Thus we could consider that most of the *P.*
256 *canariensis* populations are likely to have been subject to demographic growth and
257 the lack of statistical evidence is due to the low power of the F_S test for the most
258 recent expansions.

259

260 *Island Level: Colonisation*

261 Compared to continental areas, oceanic island populations are typically established by
262 only one or a few individual founders that successfully reproduce, leading to
263 demographic expansions. Whether the population expansions detected for *P.*
264 *canariensis* at the island level reflect the initial colonisation of the islands or
265 subsequent demographic events is difficult to know. However, times of expansion in
266 relation to the geological history of the archipelago can supply the necessary clues to
267 discern between both possibilities.

268

269 Potential maximum times for expansion are bound to the emergence times of the
270 islands. The maximum subaerial geological age of El Hierro, the youngest island, is
271 approximately one million years (Carracedo & Day, 2002). If we consider that the
272 time of the population expansion in El Hierro is $\hat{\tau} = 1.291$ and the relationship $\tau =$
273 $2\mu t$ we obtain a mutation rate estimate of 1.076×10^{-5} per locus per generation
274 (considering generation time to be 100 years as in Provan *et al.*, 1999). Using this
275 mutation rate estimate we calculated the maximum age of population expansion for
276 each sample, reported in table 2.

277

278 In order to establish a minimum time of expansion we have analysed mtDNA COII
279 sequence data for *Brachyderes rugatus* from Emerson *et al.* (2000 and unpublished
280 data). Because the niche of this species is the pine tree, its demographic expansions
281 must have occurred either during or after the establishment of the pine forest on each
282 island. Population expansions have been detected (significant F_S test) for the islands
283 of La Palma and Tenerife (138 and 182 individuals respectively, sampled throughout
284 the islands). The times of expansion for *B. rugatus* were estimated from the mismatch
285 distributions to be approximately 0.72 million years ago (mya) for Tenerife and 1.11
286 mya for La Palma (considering divergence rates to be between 2% and 2.3% per
287 million years, Brower, 1994; DeSalle *et al.*, 1987). These dates strengthen the age
288 estimates for the expansion of the pine forest obtained with the geological age
289 calibration.

290

291 These age estimates suggest expansions of the pine tree increasing in age from West
292 to East, and coinciding broadly with the colonisation ages estimated for *B. rugatus*
293 (Emerson *et al.*, 2000), as shown in table 2. We interpret the expansions at the island
294 level as a result of the colonisation process and linked to the volcanic history of the
295 archipelago. The creation of new emerged landmass by recent (up to 2 mya) volcanic
296 activity in the younger islands (La Palma and El Hierro) opened new territories for *P.*
297 *canariensis* to colonise. Note that the age of Tenerife presented in figure 2 refers to its
298 older massifs which are the remains of two or three smaller precursor islands.

299 However, the majority of the landmass of Tenerife was mainly formed by the activity
300 of Las Cañadas volcano starting around 2 million years ago (Ancochea *et al.*, 1990)
301 and it is this event which would appear to be causally related to the pine forest
302 expansion. On the island of Gran Canaria, an episode of heavy volcanic activity
303 (Roque Nublo volcano, Pérez-Torrado *et al.*, 1995) is believed to have destroyed
304 almost all terrestrial ecosystems within the island, with perhaps the exclusion of some
305 coastal regions, between 5.5 and 3 million years ago (Marrero & Francisco-Ortega,
306 2001), and this hypothesis has gained recent support from a meta-analysis by Emerson
307 (2003). The expansion of the pine forest in Gran Canaria after that event can be
308 explained either by colonisation of *P. canariensis* to the island or by a bottleneck if a
309 small pocket of pine forest survived through the Roque Nublo eruptive period.
310

311 *Sample Level: Metapopulation Dynamics*

312 The islands of the Canary archipelago have a geological history marked by recent
313 dramatic volcanic activity and giant landslides (Carracedo & Day, 2002). These
314 destructive events would have produced local elimination of pine forest, as has been
315 recorded for historical volcanic eruptions (del Arco Aguilar *et al.*, 1992; Pérez de Paz
316 *et al.*, 1994). Also, the Canary Island pine is renowned by its capacity for colonising
317 lava flows (del Arco Aguilar *et al.*, 1992; Pérez de Paz *et al.*, 1994), which suggest
318 that a metapopulation dynamic occurs within the pine forest. One of the genetic
319 signals expected in the local recolonisations after volcanic disturbances are those of
320 the demographic expansions, as it has been shown in other organisms subject to
321 similar metapopulation dynamics in other volcanic archipelagos (Beheregaray *et al.*,
322 2003; Vandergast *et al.*, 2004).
323

324 It seems very likely that local expansions detected for *P. canariensis* are the product
325 of metapopulation dynamics. When we consider different samples within the same
326 island (in Tenerife and Gran Canaria) we observe that the expansion of pine forest at
327 some areas is younger than the main demographic expansion affecting the island. We
328 hypothesize that the apparently more recent expansions may be areas recolonised after
329 geological disturbance. The role of volcanism and giant landslides in the reduction of
330 genetic diversity has also been proposed to explain the pattern of diversification of
331 *Brachyderes rugatus* in La Palma, El Hierro, Tenerife and Gran Canaria (Emerson *et*
332 *al.*, 2000).
333

334 **CONCLUSIONS**

335
336 This study demonstrates the utility of cpSSRs for the detection of demographic
337 expansions and the estimation of their relative ages. The application of population
338 genetic demographic methodology to cpSSR data for *P. canariensis* populations
339 revealed new insights into the population history of this species. The volcanic activity
340 of the archipelago appears to be a disturbance agent in the pine forest ecosystem,
341 conditioning the areas available for the pine tree. Future studies of mitochondrial
342 DNA data may further complement data from cpSSRs to elucidate the colonisation
343 and population dynamic history of *P. canariensis* on the Canary Islands. A mtDNA
344 phylogeographic analysis would reflect the historical seed movements of *P.*
345 *canariensis*, which are limited relative to pollen and may contain more fine scale
346 phylogeographic information. Additionally, a sampling design including historical
347 and isotope-dated lava flows within the pine forest may provide a good test for the
348 hypothesis of a metapopulation dynamic.

349
350 Our analyses have revealed homoplasy as a problem for the analyses (mainly in the
351 detection of younger expansions) because it reduces the power of the F_S test and
352 accuracy of absolute expansion time estimates. The development of statistics taking
353 into account the effects of homoplasy would further improve the usefulness of
354 cpSSRs as well as other linked microsatellite markers such as Y-chromosome
355 microsatellites for demographic studies.

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467 Figure 1: Coalescent process under two contrasting scenarios: constant population
468 size and sudden population expansion. For each case, the demographic history and, in
469 the same timescale measured in mutational units (1 mutational unit = $1/2\mu$
470 generations) the simulated genealogy of a random sample of genes is represented,
471 with stars representing mutational events. Below, chloroplast microsatellite mismatch
472 distribution and result of the F_S test and demographic parameters estimates for those
473 simulated samples are shown.

474
475 Figure 2: Chloroplast microsatellite mismatch distributions for the islands and map of
476 the Western Canary Islands. Maximum subaerial age of the islands (Carracedo &
477 Day, 2002) are shown in parenthesis (in million of years). Sampling localities are
478 marked with numbers, corresponding to those shown in table 2.

479 Table 1: Population expansion signal on the F_S test and mismatch distribution analysis and homoplasy level in the six simulated cases.

Case	Expansion time, τ	Mutation rate, μ		Proportion of non-significant F_S test	Proportion of significant SSD	Homoplasy index, P
		loci 1-2	loci 3-6			
1	1 (recent)	5.5×10^{-3}		0.038	0.052	0.049
2	3 (old)	5.5×10^{-5}		0.002	0.051	0.297
3	no expansion	5.5×10^{-5}		0.893	0.144	0.065
4	1 (recent)	1.65×10^{-4}	10^{-7}	0.553	0.095	0.122
5	3 (old)	1.65×10^{-4}	10^{-7}	0.240	0.060	0.606
6	no expansion	1.65×10^{-4}	10^{-7}	0.931	0.080	0.263

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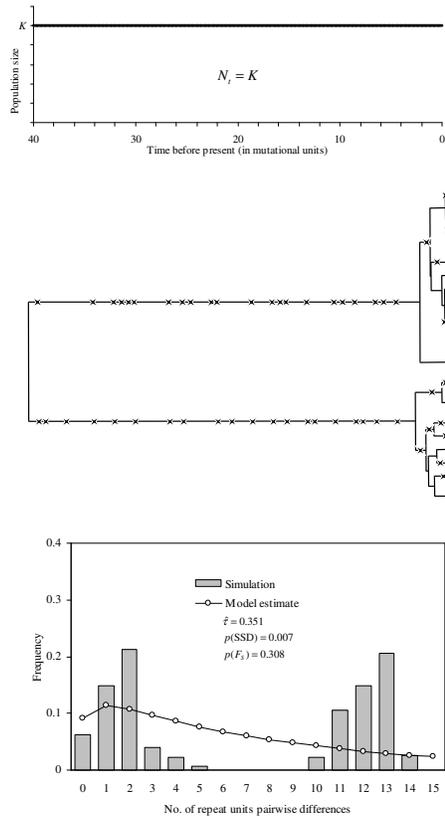
482 Table 2: Results for the F_S neutrality test for population expansion (Fu, 1997) and population expansion parameters \hat{t} following Schneider &
483 Excoffier (1999). Estimates are presented in italics when the algorithm did not converge (see Results and discussion section for details). The
484 time of expansion expressed in million of years before present (mya) is calculated using mutation rates in the range 1.076×10^{-5} per generation
485 per locus. The results for the islands (pooling samples from the same island) are presented in bold. Garabato is a monomorphic population and
486 tests could not be performed. For comparison, time for the colonisation of *Brachydes rugatus* are also presented from Emerson *et al.* (2000).

Population	N	F_S	Fu (1997) $p(F_S)$	\hat{t} (95% CI)	Schneider & Excoffier (1999) t (mya)	SSD	$p(SSD)$	Emerson <i>et al.</i> (2000) $B. rugatus$ (MYA)
Gran Canaria	145	-26.260	* < 0.001	2.544 (1.372-5.010)	1.970	0.002	0.569	> 2.56
1 Arguineguín	30	-21.890	* < 0.001	3.703 (2.138-5.636)	2.868	0.003	0.469	
2 Galdar	19	-4.076	* 0.017	2.722 (1.128-3.892)	2.108	0.004	0.554	
3 Mogán	24	-0.849	0.275	0.969 (0.000-1.558)	0.750	0.020	0.077	
4 Tamadaba	24 (23)	-1.304	0.142	0.871 (0.000-1.489)	0.675	0.014	0.138	
5 Tirajana	24	-5.052	* 0.014	1.743 (0.413-5.224)	1.350	0.008	0.399	
6 Tirma	24	-6.845	* 0.001	2.438 (0.779-3.342)	1.888	0.002	0.783	
Tenerife	280	-26.710	* < 0.001	2.374 (1.330-3.144)	1.839	0.000	0.910	1.89-2.56
7 Anaga	24	-0.192	0.185	3.000 (0.523-3.000)	2.323	0.010	0.062	
8 Arico	24	-8.983	* < 0.001	2.314 (0.675-3.185)	1.792	0.003	0.542	
9 Chinyero	50 (49)	-15.290	* < 0.001	2.294 (0.978-2.900)	1.777	0.001	0.636	
10 La Esperanza	24	-3.040	0.022	2.722 (0.483-6.413)	2.108	0.015	0.567	
11 La Guancha	24	-3.545	0.024	2.036 (0.501-2.875)	1.577	0.003	0.623	
12 Güímar	47	-22.280	* < 0.001	3.280 (1.795-4.143)	2.540	0.001	0.702	
13 Ifonche	39	-8.615	* < 0.001	2.482 (1.035-3.172)	1.922	0.007	0.130	
14 Oratava	24	-6.456	* 0.002	2.730 (0.993-6.926)	2.114	0.010	0.285	
15 Vilaflor	24	-0.165	0.437	1.081 (0.000-1.766)	0.837	0.009	0.192	
La Gomera	36	-0.424	0.427	1.558 (0.380-3.379)	1.207	0.008	0.431	
16 Garabato	12	-	-	-	-	-	-	
17 Imada	24	-0.879	0.294	1.307 (0.182-2.064)	1.012	0.002	0.711	
La Palma	48	-2.826	0.072	1.244 (0.311-1.726)	0.963	0.004	0.285	1.58-2.00
18 Fuencaliente	24	-1.063	0.224	1.289 (0.048-2.002)	0.998	0.008	0.305	

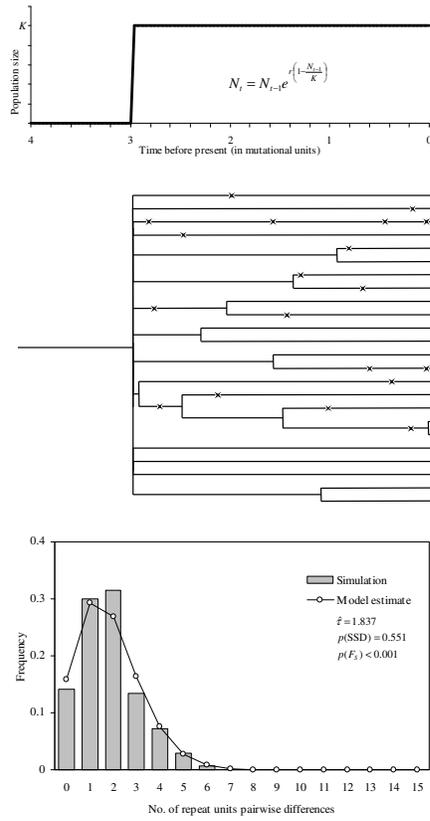
19	Garafia	24	-1.279	0.184	1.206 (0.053-1.904)	0.934	0.002	0.720
20	El Hierro	24 (23)	-3.513	* 0.008	1.291 (0.040-2.035)	1.000	0.008	0.301

487 * Significant at $\alpha = 0.05$ (p -value < 0.02)

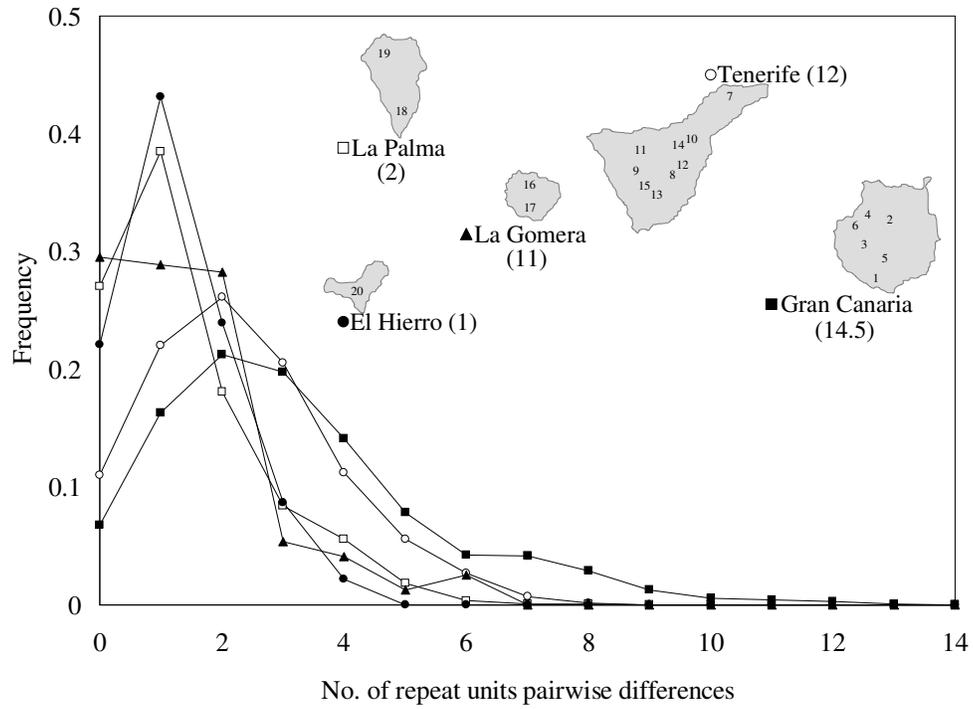
(a) Constant population size (simulation case 3)



(b) Population expansion, $\tau = 3$ (simulation case 2)



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