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1 **Natural recovery of genetic diversity by gene flow in reforested areas of the**  
2 **endemic Canary Island pine, *Pinus canariensis***

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14

15 **Abstract**

16 The endemic pine, *Pinus canariensis*, forms one of the main forest ecosystems in the

17 Canary Islands. In this archipelago, pine forest is a mosaic of natural stands (remnants

18 of past forest overexploitation) and artificial stands planted from the 1940's. The

19 genetic makeup of the artificially regenerated forest is of some concern. The use of

20 reproductive material with uncontrolled origin or from a reduced number of parental

21 trees may produce stands ill adapted to local conditions or unable to adapt in response

22 to environmental change. The genetic diversity within a transect of reforested stands

23 connecting two natural forest fragments has been studied with nuclear and chloroplast

24 microsatellites. Little genetic differentiation and similar levels of genetic diversity to

25 the surrounding natural stands was found for nuclear markers. However, chloroplast

26 microsatellites presented lower haplotype diversity in reforested stands, and this may  
27 be a consequence of the lower effective population size of the chloroplast genome,  
28 meaning chloroplast markers have a higher sensitivity to bottlenecks. Understory  
29 natural regeneration within the reforestation was also analysed to study gene flow  
30 from natural forest into artificial stands. Estimates of immigration rate into artificially  
31 regenerated forest were high (0.68–0.75), producing a significant increase of genetic  
32 diversity (both in chloroplast and nuclear microsatellites) which indicates the capacity  
33 for genetic recovery for *P. canariensis* reforestations surrounded by larger natural  
34 stands.

35

36 *Keywords:* silviculture, chloroplast microsatellites, nuclear microsatellites, assignment  
37 test, temporal samples, gene flow

38

### 39 **1. Introduction**

40 Silvicultural practices can potentially affect the genetic structure of forests and this  
41 problem is of concern for forest geneticists (see Lefèvre, 2004, for a review). In  
42 particular, artificial regeneration of forest (plantations or sowing) may produce a loss  
43 of genetic diversity through the processes of unplanned selection and genetic drift  
44 (Ledig, 1992). Detection of a reduction in genetic diversity with neutral markers  
45 because of artificial selection is unlikely because only a few genes might be involved  
46 (Lefèvre, 2004) but the quantification of genetic drift to assess potential losses of  
47 adaptive variation can be done from neutral molecular markers as it is a process that  
48 affects the whole genome (Glaubitz et al., 2003b).

49 Bottleneck effects may be present within forest regeneration practises because of the  
50 use of a limited number of seed trees and the unevenness of the number of seeds  
51 collected or produced per tree (Glaubitz et al., 2003a; Burgarella, 2004).

52

53 An additional concern for artificial populations is the extent of genetic exchange with  
54 natural populations. Potential negative effects of gene flow from artificial plantations  
55 into natural forests ('genetic pollution') have been considered (Lenormand, 2002) and  
56 have gained recent public awareness with the use of transgenic crops (including tree  
57 species, DiFazio et al., 2004). Gene flow from natural population into seed orchards  
58 (i.e. seed production plantations for reforestation) is also considered a negative effect  
59 ('pollen contamination') because it reduces the genetic gain obtained from the  
60 breeding programs and may increase maladaptation (El-Kassaby, 2000). However,  
61 under other circumstances, gene exchange between artificial and natural populations  
62 can also be positive. For small declining populations gene flow from surrounding  
63 reforestation may increase their effective population size ('demographic rescue',  
64 Lefèvre, 2004). Also, gene flow from natural forest into reforested stands may be a  
65 natural way to recover the genetic variation lost in the reforestation process. This  
66 could potentially create an admixed landrace well adapted to local conditions, and  
67 with high evolutionary potential to respond to environmental changes.

68

69 *Pinus canariensis* is endemic to the western Canary Islands, where it forms one of the  
70 main forest ecosystems of the archipelago. Its area of distribution has been diminished  
71 by five centuries of overexploitation (decreasing from covering 25% of the territory to  
72 now covering 12%). A great reforestation effort has been made since the 1940's,  
73 resulting in a mosaic of reforested and natural pine forest (del Arco Aguilar et al.,

74 1992). A high genetic differentiation has been found among populations of *P.*  
75 *canariensis* (Gómez et al., 2003) with some endangered marginal populations  
76 presenting particular genetic characteristics (Vaxevanidou et al., 2006). In the present  
77 study we analyse the genetic diversity and immigration rates within a transect of  
78 reforested stands connecting two natural forest fragments in Tenerife. Hence, we are  
79 assessing the effects of the reforestation process on the genetic integrity of the  
80 artificial stands and their potential to naturally recover to the levels of diversity of the  
81 surrounding natural forest.

82

## 83 **2. Materials and methods**

84

### 85 *2.1 Plant material and sampling design*

86 El Teide mountain (Tenerife, Spain) is crowned by a pine forest belt (the Corona  
87 Forestal Natural Park). A proportion of these pine woods are artificial stands planted  
88 from the 1940's to repair the past overexploitation (Parsons, 1981; del Arco Aguilar et  
89 al., 1992). We have chosen as our study site an area where there is still a small  
90 interruption of the forest with only a narrow strip of planted trees connecting two  
91 natural forest fragments (figure 1). Artificial *Pinus canariensis* stands were planted in  
92 Fasnía between 1956 and 1965 and in Arico between 1981 and 1985. The current  
93 vegetation cover for those areas is 30–60% for Fasnía and < 30% for Arico (del Arco  
94 Aguilar et al., 1992). The provenance of the plants is unknown but most probably seed  
95 were collected from different locations in Tenerife island (Climent et al., 1996). Nine  
96 sites were sampled in these plantations (sites 2–6 in Arico and 7–10 in Fasnía, figure  
97 1); in each of the nine sampling stations 10 planted adult trees and 10 understory  
98 naturally regenerated seedlings were sampled (except at site eight where 11 planted

99 trees and 10 seedlings were sampled). From the natural forest in Arico (site 1) and  
100 Güímar (site 11) we have sampled 50 and 47 mature trees respectively. Both old trees  
101 and seedlings were selected randomly and with an average separation of 10 m among  
102 them. From each individual needles were collected and preserved in silica gel in the  
103 summer of 2002.

104

### 105 *2.2 Molecular markers*

106 Genomic DNA was purified using a CTAB protocol based on the Doyle and Doyle  
107 (1987) method. Samples were genotyped for eight chloroplast microsatellites (Pt1254,  
108 Pt15169, Pt26081, Pt30204, Pt36480, Pt71936, Pt87268 and Pt110048; Vendramin et  
109 al., 1996) and eight nuclear microsatellites (SPAC 11.5, SPAC 11.8 and SPAG 7.14,  
110 Soranzo et al., 1998; PtTX3116 and PtTX4001, Auckland et al., 2002; *ssrPt\_ctg4363*,  
111 *ssrPt\_ctg4698* and *ssrPt\_ctg7731*, Chagné et al., 2004). PCR amplifications were  
112 performed in a Perkin-Elmer 9700 thermal cycler (details in Navascués, 2005). PCR  
113 products were sized using an ABI PRISM 3700 DNA Analyzer (Applied Biosystems).

114

### 115 *2.3 Genetic diversity indices*

116 For chloroplast microsatellites, total number of haplotypes ( $n_h$ , direct count of  
117 haplotypes which are scored as unique combinations of alleles for all cpSSR loci),  
118 effective number of haplotypes ( $n_e$ , Nielsen et al., 2003), unbiased haplotype diversity  
119 ( $H_e$ , Nei, 1978) and average genetic distances among individuals ( $D_{sh}^2$ , Goldstein et  
120 al., 1995) were calculated for each sample:

$$121 \quad n_e = (n-1)^2 \left/ \sum_{h=1}^{n_h} p_h^2 \right. (n+1)(n-2) + 3 - n \quad [1]$$

$$122 \quad H_e = \frac{n}{n-1} \left( 1 - \sum_{h=1}^{n_h} p_h^2 \right) \quad [2]$$

123 
$$D_{sh}^2 = \frac{2}{n(n-1)} \frac{1}{L} \sum_{i=1}^n \sum_{j=i+1}^n \left( \sum_{k=1}^L |a_{ik} - a_{jk}| \right)^2 \quad [3]$$

124 where  $n$  is the number of individuals in the sample,  $p_h$  is the relative frequency of the  
 125  $h^{\text{th}}$  haplotype,  $n_h$  is the number of different haplotypes in the sample,  $L$  is the number  
 126 of loci,  $a_{ik}$  is the size (number of repeats) of the allele for the  $i^{\text{th}}$  individual and at the  
 127  $k^{\text{th}}$  locus, and  $a_{jk}$  is the size of the allele for the  $j^{\text{th}}$  individual and at the  $k^{\text{th}}$  locus.

128

129 For nuclear microsatellites the arithmetic mean among loci was calculated for the  
 130 following indices: number of alleles ( $A$ , direct count), effective number of alleles ( $A_e$ ,  
 131 Nielsen et al., 2003), and unbiased gene diversity ( $H_e$ , Nei, 1978):

132 
$$A_e = (2n-1)^2 / \sum_{i=1}^A p_i^2 (2n+1)(2n-2) + 3 - 2n \quad [4]$$

133 
$$H_e = \frac{2n}{2n-1} \left( 1 - \sum_{i=1}^A p_i^2 \right) \quad [5]$$

134 where  $n$  is the number of individuals in the sample (i.e. sample size),  $p_i$  is the relative  
 135 frequency of the  $i^{\text{th}}$  allele in the sample and  $A$  is the total number of different alleles in  
 136 the sample.

137

138 Significant differences in the genetic diversity indices between pairs of samples were  
 139 tested by a Monte Carlo approach (see, for instance, Glaubitz et al., 2003a for similar  
 140 analysis) programmed in Fortran language (source code available from the authors on  
 141 request). The null distribution (both samples hold the same genetic diversity) of  
 142 differences for each index was constructed by resampling (with replacement)  
 143 individuals among samples. The result is a mixture of individuals from both samples  
 144 distributed in two new samples (with sizes equal to the original sizes). The genetic  
 145 diversity parameters of both samples and their differences are computed (samples are

146 compared always in the same order and sign of differences is kept). This process is  
147 repeated 10 000 times to build the null distribution of the genetic diversity differences  
148 expected for two samples (with the specific sizes of the empirical samples) drawn  
149 from populations with the same genetic diversity (i.e. the same population). The  
150 empirical difference value is then compared with the null distribution. Since we are  
151 interested in testing for the reduction of genetic diversity (i.e. null hypothesis,  $h_0$ :  
152 diversity in natural forest  $\leq$  diversity in artificial stands) we perform a one-tailed test.  
153 The  $p$ -value for rejecting the null hypothesis is estimated as the proportion of  
154 iterations with differences greater than or equal to the observed difference value. This  
155 process is used to test chloroplast haplotype diversity, individual nuclear locus  
156 diversity and average (among loci) nuclear diversity. Two types of comparisons were  
157 made, 1) samples from the natural forest with samples from the trees planted in the  
158 artificial stands and 2) samples from the trees planted in the artificial stands with the  
159 natural regeneration in the artificial stands.

160

161 Significant differences in allele frequencies (nSSR) between pairs of samples were  
162 tested with a Fisher exact test (Raymond and Rousset, 1995) using the program  
163 Genepop 3.4 (available at <http://wbiomed.curtin.edu.au/genepop/>).

164

#### 165 *2.4 Temporal change in allelic frequencies*

166 Allele frequencies change through time through the influences of gene flow and  
167 genetic drift (assuming neutral variation). Using the change in allele frequencies in  
168 samples of different generations has been mainly used for estimating effective  
169 population sizes (e.g. Waples, 1989). Recently Wang and Whitlock (2003) have  
170 developed a new maximum likelihood method that estimates immigration rates and



171 effective population sizes simultaneously. This method, implemented in the MLNE  
172 program (available at <http://www.zoo.cam.ac.uk/ioz/software.htm>), has been used on  
173 the nSSR data of the reforested samples using the planted trees as the parental  
174 generation and the natural regeneration as the following generation. To estimate the  
175 gene flow, the method also uses the allele frequencies of the potential external source  
176 of genes, which in our case were taken from the surrounding natural stands.

177

### 178 *2.5 Additional analysis of gene flow*

179 A hierarchical AMOVA on cpSSRs was used to study the paternal heterogeneity from  
180 the sampled seedlings, as in Dyer and Sork (2001). The chloroplast is paternally  
181 inherited in conifers; therefore, cpSSR variation in the seedlings might reflect the  
182 genetic diversity of the pollen clouds of the area. However, in our case, having  
183 sampled seedlings on the ground, we cannot ignore some influence of seed dispersal  
184 in our data. Canopy density can influence the dispersal of propagules diminishing  
185 windspeed (see Nathan and Katul, 2005). Thus, sampling sites were nested into two  
186 AMOVA groups, Arico (low vegetation cover) and Fasnica (high vegetation cover, del  
187 Arco Aguilar et al., 1992).

188

189 The genotype assignment method developed by Rannala and Mountain (1997),  
190 implemented in their program Immanc 5 (available at  
191 <http://www.rannala.org/labpages/software.html>), was used on the nSSR data to detect  
192 immigrants among the seedlings. This method allows performing the test for different  
193 levels of immigrant ancestry considering the probabilities of external origin of: A) the  
194 whole genotype (first generation immigrant, i.e. the individual has two foreign  
195 parents), B) half of the genotype (second generation immigrant, i.e. the individual has

196 one foreign parent), etc. The program calculates the relative probability for each  
197 individual to be born from parents with no recent immigrant ancestry rather than from  
198 both foreign parents (denoted  $\Lambda_{d=0}$ ) or from one foreign parent (denoted  $\Lambda_{d=1}$ ).  
199 These calculations are based on the allele frequencies of the studied population and  
200 the potential source populations using a Bayesian approach. The null distribution  
201 (individual with no immigrant ancestry) of the statistic  $\ln \Lambda$  is built by a Monte Carlo  
202 simulation and  $p$ -values and statistical power (for  $\alpha = 0.05$ ) are estimated for each test.  
203

204 In our case the interpretation of the Rannala and Mountain (1997) assignment tests are  
205 slightly different, because we have effectively only one generation of possible  
206 immigrants. ‘First generation’ immigrants (whole genotype of external origin) will  
207 correspond to seeds developed and fertilised outside the stand. ‘Second generation’  
208 immigrants (half genotype of external origin) will correspond to seeds from the stand  
209 fertilised with pollen from outside (the alternative possibility, seed from outside  
210 fertilised with pollen from the stand and dispersed into the stand, is considered highly  
211 improbable and will be ignored in the interpretation). The proportion of ‘seed-  
212 immigrants’ ( $d = 0$ ) and ‘pollen-immigrants’ ( $d = 1$ ) in the analysed seedlings will be  
213 estimates of seed and pollen immigration rates. To check the accuracy of the method  
214 we examined the results of these tests on the planted trees, which we consider by  
215 definition ‘non-immigrants’. The proportion of planted trees detected as immigrants  
216 will give us the error rate of the method.

217

### 218 **3. Results and discussion**

#### 219 *3.1 Genetic differences among samples*

220 Significant differentiation ( $\chi^2 = 50.438$ , d.f. = 16,  $p < 0.001$ ) in the nSSR allele  
221 frequencies was detected between planted and natural forest. However these  
222 differences cannot be interpreted as introduction of foreign material as significant  
223 differentiation ( $\chi^2 = 54.787$ , d.f. = 16,  $p < 0.001$ ) was also detected between the two  
224 spatially close natural stands. Also, there were significant differences in the allele  
225 frequencies between planted trees and understory natural regeneration for both  
226 reforested stands (Arico:  $\chi^2 = 27.282$ , d.f. = 16,  $p = 0.038$  and Fasnica:  $\chi^2 = 28.005$ , d.f.  
227 = 16,  $p = 0.032$ ). These changes may be the result of the effects of drift and gene flow  
228 in the reforested stands (see next section).

229

230 Levels of nSSR genetic diversity in the reforested stands are, in general, similar to  
231 those found in the surrounding natural forest (table 1, results for individual nSSR loci  
232 not shown). Mean expected heterozygosity presented a significantly lower value in  
233 the plantations but the diversity index based on allele number, which is more sensitive  
234 to population bottlenecks (Nei et al., 1975), showed no significantly lower values and  
235 in some cases allele diversity was even higher. Therefore, it is unclear what the lower  
236  $H_e$  value indicates. The higher proportion of rare alleles (alleles with frequency  $<$   
237 0.01) in the reforested stands (27.45%) in comparison to the natural forest (19.42%)  
238 suggests to us that a possible mixed origin of the seedling stock (or different seedling  
239 origins for the different reforestation phases) could produce an accumulation of alleles  
240 from different areas (explaining high levels of allelic diversity) but with lower  
241 frequencies in the mixture (resulting in low  $H_e$  values). In fact, observed  
242 heterozygosity (mean  $H_o=0.648$ ) is lower than the expected heterozygosity suggesting  
243 the possibility of a Wahlund effect ( $F_{IS}=0.112$ ,  $p$ -value = 0.006).

244

245 For cpSSRs, there is a significantly lower effective number of haplotypes in both  
246 artificial stands compared to natural forest (table 1). Bottlenecks associated with the  
247 reforestation process could have had a stronger effect on cpSSR diversity than in  
248 nSSRs because the effective population size for the chloroplast genome is half the  
249 nuclear effective population size. However, no significant reduction was found at  $H_e$   
250 and  $D^2_{sh}$ , probably because these measures are less sensitive to population size  
251 changes. Alternatively, the lower haplotype diversity found could be attributed to a  
252 low genetic diversity provenance for the seedlings used in the plantation. However,  
253 low diversity populations of *P. canariensis* are mainly marginal (Vaxevanidou et al.  
254 2006) which are unlikely locations for seed collection by Forest Services.

255

256 In the comparison of the reforested areas with their natural regeneration we found a  
257 significant increase in the effective number of haplotypes and in the mean effective  
258 number of alleles (table 2). Although the increase on mean  $A_e$  was not apparent when  
259 the two reforested stands were studied separately, the whole reforestation area had a  
260 significant gain in alleles for loci SPAC 11.8, PtTX3116, PtTX4001, ssrPt\_ctg4698  
261 and ssrPt\_ctg7731 (data not shown). This increase of the allelic diversity in the  
262 reforestation may be attributable to gene flow from the natural stands.

263

### 264 *3.2 Gene flow into the artificial stands*

265 Three different methods have been used to explore the immigration dynamics within  
266 the reforested stands. First, we examined the temporal changes in allele frequencies  
267 between the planted trees and the following generation, represented by the understory  
268 natural regeneration. We applied the Wang and Whitlock (2003) maximum likelihood  
269 method using the allele frequencies in the natural forest as the potential external

270 source of immigrants. The estimates (and 95% confidence intervals) of effective  
271 population size ( $N_e$ ) and immigration rate ( $m$ ) obtained from this method are  $N_e =$   
272 36.88 (29.09–50.12) and  $m = 0.68$  (0.46–0.88) for Arico and  $N_e = 28.83$  (22.95–  
273 38.78) and  $m = 0.75$  (0.54–0.94) for Fasnja.

274

275 Two other methods were employed to characterize gene flow in the area but none of  
276 them shed further light on the subject. The results of the AMOVA test gave non-  
277 significant differentiation of the pollen diversity for both among sites and among  
278 stands ( $\Phi$ -statistics  $< 0.033$ ,  $p > 0.17$ ). This result suggests a high pollen dispersal  
279 capacity. A second method was the estimation of immigration rates by the  
280 identification of immigrants among the natural regenerated seedlings with the Rannala  
281 and Mountain (1997) assignment test. The immigration rates into the reforested  
282 patches, calculated as the proportion of identified immigrants over the sample size,  
283 are  $m_{seed} = 0.07$  and  $m_{pollen} = 0.12$ . However, these estimates are unreliable as when  
284 the same assignment test was applied to the planted trees (considered ‘non-  
285 immigrants’) 15 individuals were identified as second generation immigrants (pollen  
286 dispersal) and 6 as first generation immigrants (seed dispersal). These error rates are  
287 in the same order of magnitude as the estimated immigration rates obtained. This poor  
288 performance of the test is due to the low genetic differentiation among the samples  
289 which resulted in very low statistical power (results not shown).

290

291 The high levels of gene flow suggested by these results are not surprising as pines are  
292 predominantly outcrossing and wind pollinated (Ledig, 1998). Previous estimates of  
293 immigration rates in artificial stands are available from pollen contamination studies  
294 in seed orchards. Among the factors influencing the pollen contamination levels in

295 seed orchards are the distance to the nearest stands of the same species and the  
296 relative pollen production of the seed orchard to the surrounding forest (Adams and  
297 Burczyk, 2000). Wind direction can also increase pollen contamination locally  
298 (Yazdani and Lindgren, 1991). The range of pollen immigration rates found for pines  
299 in such studies are high, ranging from 0.26 to 0.75 (both estimates for *Pinus sylvestris*  
300 seed orchards, Harju and Muona, 1989; Yazdani and Lindgren, 1991) depending on  
301 the particulars of each case. Immigration rates calculated in natural stands from  
302 paternity analysis are also high 0.30–0.31 (minimum estimates, Lian et al., 2001;  
303 González-Martínez et al., 2003) but decrease to 0.05–0.07 in isolated populations  
304 (Schuster and Mitton, 2000; Robledo-Arnuncio and Gil, 2004). Therefore, the close  
305 proximity to big natural forest and the small size of the reforested stands studied  
306 offers some explanation for the high levels of immigration into the reforested area.

307

#### 308 **4. Conclusions**

309 The effect of the reforestation process was detected in the chloroplast genome with a  
310 reduction in the effective number of haplotypes while the effects on the nuclear  
311 genome were uncertain. The difference in the effective population size between the  
312 two genomes could explain a different sensitivity to bottlenecks. These results suggest  
313 that, for the particular case studied, the planted stock originated from a reduced  
314 number of trees. Despite the lower haplotype diversity found within the plantations  
315 the richness of their future genetic pool does not seem jeopardized because both  
316 chloroplast and nuclear genetic diversities appear to increase within the understory  
317 natural regeneration. High levels of gene flow from the adjacent and larger natural  
318 stands explain this rise in diversity. These results highlight the importance of the  
319 presence and abundance of natural forest for the regeneration of genetically rich forest

320 (see, for example, Glaubitz et al., 2003a; Glaubitz et al., 2003b). However, these  
321 conclusions should not be extended to other *P. canariensis* plantations, many of  
322 which are located far from natural stands (such as those found on north Tenerife, del  
323 Arco Aguilar et al., 1992). Current management of these reforested areas is mainly  
324 focused on the reduction of the number of trees to facilitate natural regeneration (Gil,  
325 2006). This present work suggests the utility of studying the genetic makeup of  
326 artificial stands and their present or future natural regeneration. This may allow for  
327 the assessment of whether the reforestation process reduces genetic diversity (which  
328 will depend on the particular reforestation management done at different times and  
329 places) and, for those stands affected, their potential to recover genetic diversity in the  
330 following generations. This may identify stands for which the plantation of new  
331 individuals, from a controlled provenance and originating from numerous parental  
332 trees, may be beneficial for increasing their pool of genetic diversity.

333

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337

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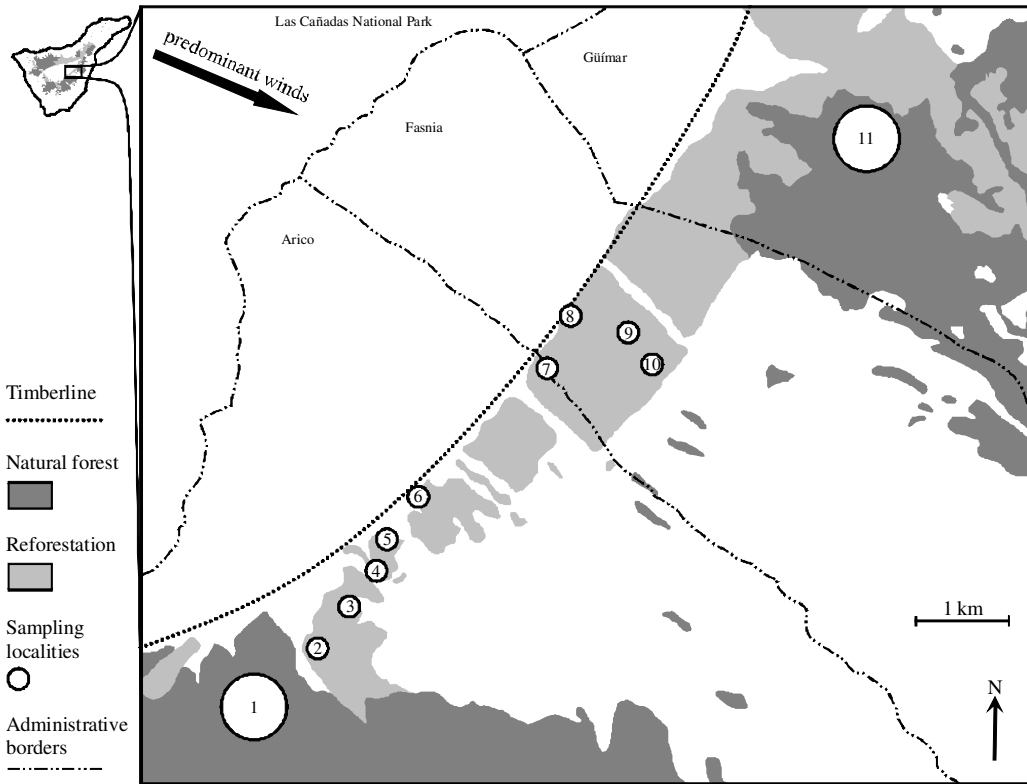
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443

444 *Figure 1.*— Map of the study site on the South-eastern slope of Tenerife (Canary  
 445 Islands). Sampling locations are marked with white circles proportional in diameter to  
 446 the sampling size.

447

448 *Table 1.*— Diversity indices in the natural forest (pooled Arico and Güímar samples)  
 449 and reforested areas (with statistical significance of the difference with natural forest  
 450 in parentheses).

		Natural	Planted, both stands ( <i>p</i> -value)	Arico, planted trees ( <i>p</i> -value)	Fasnia, planted trees ( <i>p</i> -value)
	Sample size	97	91	50	41
chloroplast	$n_h$	49	46 (0.312)	32 (< 0.001*)	25 (< 0.001*)
	$n_e$	34.496	19.051 (0.006*)	26.085 (0.056)	12.828 (< 0.001*)
	$H_e$	0.971	0.948 (0.142)	0.962 (0.287)	0.923 (0.115)
	$D^2_{sh}$	3.120	2.739 (0.261)	2.584 (0.156)	2.984 (0.461)
	mean $A$	17.735	19.125 (0.990)	15.875 (0.895)	13.875 (0.340)
nuclear	mean $A_e$	9.618	9.335 (0.360)	10.493 (0.832)	8.583 (0.128)
	mean $H_e$	0.753	0.729 (0.032*)	0.726 (0.038*)	0.733 (0.119)

451 \* Significant at  $\alpha = 0.05$

452

453 Table 2.— Diversity indices in the reforested areas and their understory regeneration,  
 454 and statistical significance of the differences. Analysis done for the whole reforested  
 455 area, reforested area in Arico (planted in 1981-85) and reforested area in Fasnía  
 456 (planted in 1956-65). Sample sizes in parentheses.

		Planted (91)	Regeneration (90)	<i>p</i> -value
chloroplast	$n_h$	46	49	0.219
	$n_e$	19.051	35.768	0.006*
	$H_e$	0.948	0.972	0.193
	$D^2_{sh}$	2.739	3.033	0.322
	mean $A$	19.125	19.375	0.342
nuclear	mean $A_e$	9.335	11.100	0.012*
	mean $H_e$	0.729	0.760	0.027*
		Arico planted (50)	Arico regeneration (50)	<i>p</i> -value
chloroplast	$n_h$	32	36	0.106
	$n_e$	26.085	47.152	0.001*
	$H_e$	0.962	0.980	0.211
	$D^2_{sh}$	2.584	3.194	0.163
	mean $A$	15.875	14.250	0.949
nuclear	mean $A_e$	10.493	10.511	0.489
	mean $H_e$	0.726	0.738	0.213
		Fasnía planted (41)	Fasnía regeneration (40)	<i>p</i> -value
chloroplast	$n_h$	25	24	0.689
	$n_e$	12.828	22.314	0.037*
	$H_e$	0.923	0.956	0.228
	$D^2_{sh}$	2.984	2.771	0.573
	mean $A$	13.875	13.750	0.498
nuclear	mean $A_e$	8.583	9.723	0.122
	mean $H_e$	0.733	0.736	0.438

457 \* Significant at  $\alpha = 0.05$