

CANARY ISLANDS PINE (*PINUS CANARIENSIS* CHR. SM. EX DC.) 2. GENE FLOW AMONG NATIVE POPULATIONS*

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ABSTRACT

Gene flow (Nm), expressed as number of migrants, was estimated by means of three different models. The regression between Nm and the geographic distance was non significant; still two groups of vectors which differ one from the other do exist: the one with high gene flow directed from El Hierro to Gomera (8.33), El Hierro to La Palma (4.79) and Gomera to La Palma (3.95); the second group, with relatively low gene flow between 2.42 and 3.05 is directed between the other pairs of islands. The total level of gene flow in *P. canariensis* was estimated as 2.52.

Keywords: *Pinus canariensis*, gene-flow.

INTRODUCTION

The Canary Island pine (*Pinus canariensis* Chr. Sm. ex DC.) populations that grow on each of the five Canary Islands (*i.e.*, Tenerife, La Palma, Gran Canaria, Gomera, El Hierro), four of which are situated in a circle of about 100 km in radius from the island of Tenerife, and are between 27 to 84 km apart, differ among themselves according to islands, altitude and ecological zonation in the number of polymorphic loci and in the number of private alleles (SCHILLER *et al.*, this issue). This is probably the result of restricted gene flow as has been shown for many conifers (HAMRICK 1987; GOVINDARAJU 1988; ELLSTRAND 1992; GONCHARENKO *et al.* 1993a, 1993b, 1994; KIM & LEE 1995; YANG & YEH 1995).

Gene flow influences the genetic differentiation of species and populations within species. High rates of gene flow among populations smooths the effects of genetic drift and selection, which on the other hand create new gene combinations, which help the species to adapt to new conditions, *i.e.*, microgeographic differentiation (WRIGHT 1951; CROW & AOKI 1984; SLATKIN 1985, 1987). WRIGHT's (1951) island model assumes that every local population is separated from every other one, estimations of gene flow are based on this postulate and are calculated as if each of the populations were independent. In nature, however,

neighbouring populations may exchange migrants between themselves. The stepping stone model estimates gene disperse which occurs only between populations (CROW & AOKI 1984; SLATKIN 1993). For estimation of gene flow between populations in that model, alleles which occurred only in one sampled population, *i.e.*, unique alleles (= private alleles) are used. The average frequency of private alleles across populations is a sensitive predictor of gene flow (SLATKIN 1985, 1987; SLATKIN & BARTON 1989).

The aim of the present study was to analyze the level of gene flow, rates of migration between populations of *P. canariensis* and among the five islands.

MATERIALS AND METHODS

This article is based on the seed material and the results gained by electrophoresis which were described in the former article in this volume.

Data analysis

Standard measures of genetic variation were calculated at the population, ecological groups, island and species level (SCHILLER *et al.* 1999). Data of allele frequency were used to calculate G_{ST} values (NEI 1973, 1978). Total gene differentiation (H_t) and mean gene differentiation within populations (H_s) were calculated using

the GENSTAT computer program (LEWIS & WHITKUS 1989). Genetic distance (D) were calculated for all pairs of populations (NEI 1978).

The F_{ST} values (WRIGHT 1978) were calculated using the POPGEN computer program (YEH *et al.* 1997), and were used for the estimation of gene flow $Nm_{(W)}$ that represent the level of gene flow in an island model. Slatkin's private allele method (SLATKIN 1985) which assumes that the frequency of an allele that occurs in one population is proportional to the gene flow among populations, was used to estimate the gene flow $Nm_{(S)}$. Estimation of gene flow among sub-populations [$Nm_{(C)}$] (*i.e.*, gene flow among several populations on one island or ecological group) was done using Crow's formula (CROW & AOKI 1984).

The significant of excess or deficiency of heterozygotes was tested using the Li formula (LI & HORVITZ 1953; NEI 1977). The time divergence between isolated populations was estimated according to Nei's method (NEI 1975).

RESULTS

The number of trees analyzed in each of the 22 population, mean number of alleles per loci (A), within-population gene differentiation (H_s), the number of

unique alleles (= private alleles) (U), the mean frequency of unique alleles ($P_{(1)}$) and the gene flow $Nm_{(S)}$ are presented in Table 1. Private alleles were detected in 18 out of the 22 populations analyzed. Unique allele frequencies ranged between 0.000 to 0.145, as the result gene flow $Nm_{(S)}$ ranged from 0.00 to 11.96. At the species level, mean number of alleles per loci, within-population gene differentiation and mean number of private alleles was 1.76, 0.122 and 1.95, respectively. Therefore, mean $Nm_{(S)}$ was estimated to be 3.08.

Nei's genetic distances between the islands were small (Table 2), this low levels of differentiation may reflect also the small geographic distances between the islands, although the regression between genetic and geographic distances proved to be non-significant. Mean values of genetic distance within six ecological zones are: on the island of Tenerife, 1A – 0.0066 (0.0053–0.0078), 1B – 0.0060 (0.0029–0.0066), 1C – 0.0214. On the island of La Palma: 2A – 0.0039 (0.0082–0.0098), 2B – 0.0046 (0.0028–0.0068). On the island of Gran Canaria 0.0161 (0.0094–0.0291).

Gene flow (Nm), as number of migrants, was estimated using three different models of population gene migration: (a) the island model (WRIGHT 1951) based on the equilibrium relationship; (b) the private allele method (SLATKIN 1985) based on the frequency

Table 1. Genetic variability and gene flow of *Pinus canariensis* at 22 populations.

Island	Pop No.	Name	N	A	H_s	U	$P_{(1)}$	$Nm_{(S)}$
Tenerife	0	Arafo	18	1.75	0.124	1	0.028	5.29
	1	La Orotava	17	1.69	0.110	1	0.029	4.99
	2	La Guancha	15	1.69	0.121	0	0.000	–
	3	Garachico	23	1.88	0.135	2	0.032	4.26
	4	Vilafior	31	1.91	0.121	1	0.048	2.19
	5	Adeje	21	1.69	0.096	1	0.024	6.81
	6	Arico	30	2.00	0.136	1	0.017	11.96
	7	Candelaria	13	1.38	0.076	0	0.000	–
	8	La Esperanza	12	1.69	0.143	2	0.042	2.73
La Palma	9	La Laguna	9	1.63	0.141	1	0.056	1.71
	10	Punta Llana	18	1.84	0.133	0	0.000	–
	11	Barlovento	10	1.72	0.141	4	0.063	1.41
	12	Garafia	27	1.78	0.114	2	0.028	5.29
	13	Punta Gorda	21	1.94	0.140	4	0.030	4.29
	14	El Paso	30	1.78	0.101	2	0.017	11.96
El Hierro	15	Fuencaliente	28	1.78	0.135	0	0.000	–
	16	San Salvador	29	1.84	0.121	4	0.031	4.48
Gran Canaria	18	Tamadaba	21	1.78	0.106	4	0.042	2.73
	19	Tirma	27	1.78	0.141	3	0.136	0.40
	20	Tejeda	28	1.84	0.116	4	0.022	7.85
	21	Mogan	30	1.88	0.129	4	0.025	6.37
Gomera	22	Garabato	7	1.41	0.112	2	0.145	0.36
<i>P. canariensis</i>		Species level		1.76	0.122	1.95	0.039	3.08

Note: N – number of trees analyzed; A – mean number of alleles per locus; H_s – within-population gene differentiation; U – number of unique alleles; $P_{(1)}$ – mean frequency of unique alleles.

Table 2. Genetic distances between island populations of *P. canariensis*.

Islands	Tenerife	La Palma	El Hierro	Gran Canaria	Gomera
Tenerife	***				
La Palma	0.0064	***			
El Hierro	0.0110	0.0022	***		
Gran Canaria	0.0081	0.0036	0.0040	***	
Gomera	0.0121	0.0061	0.0029	0.0057	***

Table 3. Gene flow and levels of genetic variability of *Pinus canariensis* at five islands.

Island	<i>N</i>	<i>N_p</i>	<i>H_s</i>	<i>P</i>	<i>U</i>	<i>P₍₁₎</i>	<i>Nm_(s)</i>	<i>F_{ST}</i>	<i>Nm_(w)</i>	<i>Nm_(c)</i>
La Palma	146	6	0.127	21	12	0.023	7.30	0.051	4.65	3.23
Tenerife	189	10	0.120	25	10	0.028	5.42	0.074	3.13	2.53
Gran Canaria	106	4	0.123	23	15	0.056	1.71	0.085	2.69	1.51
El Hierro	29	1	0.121	16	4	0.031	4.48	–	–	–
Gomera	7	1	0.112	11	2	0.145	0.36	–	–	–
Species level	465	22	0.122	28	17	0.039	3.08	0.91	2.497	2.275

Note: *N* – number of trees analyzed; *H_s* – within population gene differentiation; *P* – number of polymorphic loci; *U* – number of unique alleles; *P₍₁₎* – mean frequency of unique alleles; *Nm_(s)* – gene flow based on Slatkin's method; *F_{ST}* – genetic diversity; *Nm_(w)* – gene flow according to Wright's method; *Nm_(c)* – gene flow according to Crow's method.

of an allele that occurs only in one population; and (c) stepping stone model (CROW & AKOI 1984) in which genes are exchanged between neighbouring populations only.

According to the island model, all populations on each of the three larger Islands were pooled together, and genetic diversity (*F_{ST}*) among them was calculated (Table 3). The *F_{ST}* calculated for the island of Gran Canaria was 0.085, for Tenerife – 0.074, and for La Palma – 0.051. These *F_{ST}* values were used to estimate the gene flow among populations on each of the islands, *i.e.*, *Nm_(w)* which resulted to be 2.69 for Gran Canaria, 3.13 for Tenerife and 4.65 for La Palma. Gene flow was not estimated for the islands of El Hierro and Gomera because of the very small sample sizes. Calculations of gene flow using the two other methods did not revealed the same results. *Nm_(s)* according to SLATKIN (1985) were much higher, and could be calculated also for the islands of El Hierro and Gomera. *Nm_(c)* calculations according to CROW & AKOI (1984) resulted in similar results to *Nm_(w)*. Still, with the exception of El Hierro and Gomera, the same trend in the reduction of gene flow from La Palma to Gran Canaria via Tenerife was observed. The large differences in the results of gene flow amount calculated according to WRIGHT (1951) or CROW & AKOI (1984) and those calculated according to SLATKIN (1985) are probably because Slatkin's method depends critically on the sample size. However, almost all *Nm* obtained were

greater than 1.0, which means that gene flow would prevent substantial differentiation due to genetic drift.

The island of Tenerife was divided into three ecological units, La Palma into two ecological units and Gran Canaria only one ecological unit (CLIMENT-MALDONADO *et al.* 1996). Gene flow for each of the ecological units, or for the three altitudinal zones of 500 to 1000; 1001 to 1500 and 1501 to 2100 m a.s.l. were calculated, the results are presented in Table 4. For each of the ecological units, gene flow differed according to the method used; whereas, for each of the higher zones, gene flow was similar.

The estimated values of gene flow for each pair of Islands (Table 5), was calculated from the pairwise value of *F_{ST}*, that indicate the amount of gene flow between them. Gene flow was computed for 10 pairs, 10(5*4/2), values ranged from 2.42 to 8.33. Gene flow according to Crow and Akoi ranged between 1.579 and 3.490; and according to Slatkin's method – between 2.720 and 35.907.

DISCUSSION

Low levels of genetic differentiation of allozyme loci between populations can be explained by wind-pollination, seed dispersal by wind and by birds, that reduce the influence of genetic drift and therefore, decreases the heterogeneity of allele frequencies and intergenetic differentiation (HAMRICK *et al.* 1981; HAMRICK &

Table 4. Gene flow and levels of genetic variability of *Pinus canariensis* according to ecological and altitudinal zones.

Ecological zone	Island	<i>N</i>	<i>U</i>	$P_{(1)}$	$Nm_{(s)}$	F_{ST}	$Nm_{(w)}$	$Nm_{(c)}$	H_s	<i>P</i>
1A	Tenerife	55	3	0.020	8.93	0.039	6.160	2.738	0.129	21
1B	Tenerife	85	3	0.030	4.73	0.031	7.815	3.473	0.124	23
1C	Tenerife	25	2	0.021	8.46	0.090	2.528	0.632	0.119	18
2A	La Palma	55	6	0.030	4.73	0.050	4.750	2.111	0.131	23
2B	La Palma	82	6	0.016	13.20	0.028	8.679	3.857	0.127	23
4	Gran Canaria	106	15	0.056	1.71	0.085	2.691	1.512	0.123	23
No of populations	Altitude	<i>N</i>	<i>U</i>	$P_{(1)}$	$Nm_{(s)}$ *	F_{ST}	$Nm_{(w)}$	$Nm_{(c)}$	H_s	<i>P</i>
7	500–1000	151	20	0.064	2.53	0.091	2.497	1.835	0.128	24
10	1001–1500	219	14	0.021	2.64	0.081	2.836	2.298	0.118	27
5	1501–2100	98	7	0.029	2.08	0.064	3.656	2.340	0.123	24

Note: *N* – number of trees analyzed; H_s – within population gene differentiation; *P* – number of polymorphic loci; *U* – number of unique alleles; $P_{(1)}$ – mean frequency of unique alleles; $Nm_{(s)}$ – gene flow based on Slatkin's method; F_{ST} – genetic diversity; $Nm_{(w)}$ – gene flow according to Wright's method; $Nm_{(c)}$ – gene flow according to Crow's method; $Nm_{(s)}$ * – average gene flow in group according to Slatkin's method.

Table 5. Geographic distance between pairs of islands (Dist.), genetic diversity (F_{ST}), and gene flow between island populations of *Pinus canariensis*.

Island I	Island II	N_{pop}	Geogr. dist.	F_{ST}	$Nm_{(w)}$	$Nm_{(c)}$	$Nm_{(s)}$
Gran Canaria	Tenerife	10/4	60	0.093	2.44	2.102	4.073
Gran Canaria	Gomera	4/1	95	0.092	2.42	1.579	–
Gran Canaria	La Palma	6/4	198	0.076	3.05	2.462	7.855
Gran Canaria	Hierro	1/4	202.5	0.078	2.97	1.891	–
Tenerife	Gomera	10/1	27	0.084	2.72	2.253	11.214
Tenerife	La Palma	10/6	84	0.081	2.84	2.493	35.907
Tenerife	Hierro	10/1	112.5	0.078	2.97	2.442	2.720
La Palma	Gomera	6/1	55.5	0.060	3.95	2.878	–
La Palma	Hierro	6/1	67.5	0.050	4.79	3.490	–
Hierro	Gomera	1/1	61.5	0.029	8.33	2.093	14.142

Note: *N* – number of local populations; F_{ST} – genetic diversity; $Nm_{(s)}$ – gene flow based on Slatkin's method; $Nm_{(w)}$ – gene flow according to Wright's method; $Nm_{(c)}$ – gene flow according to Crow's method.

GODT 1989; HAMRICK *et al.* 1992). We have found a slight decreases in differentiation (F_{ST}), among islands (Table 3).

Genetic differentiation among natural populations of rather small size may arise from major mechanisms such as random genetic drift, mutations and selection; gene flow may reduce the effect of this mechanisms. In the island model if gene flow $Nm = 1$, that is enough to keep the same alleles in all populations, if $Nm > 1$, then gene flow is strong enough to prevent their genetic differentiation due to genetic drift (SLATKIN 1985). Genetic drift and selection may be a significant factors for local differentiation that can be observe on the Canary Islands. WRIGHT (1951) showed that at drift-migration equilibrium, the average number of migrants per population and per generation (Nm) is inversely

proportional to the levels of genetic differentiation among population, measured as the F_{ST} . This relationship is based on the assumption that gene flow is uniform among populations. We estimated Nm among all populations of *P. canariensis*, within Islands, and between pairs of Islands. Using Wright's method the total level of gene flow $Nm_{(w)}$ among populations of *P. Canariensis* is 2.5, which is quite low for a wind pollinated species in comparison with other pine species. Most conifers have extensive gene flow *e.g.*, $Nm = 5.56$ in *P. pumila*; – 8.68 in *P. sibirica*; – 6.2 in *P. sylvestris* (GONCHARENKO *et al.* 1993a, 1993b, 1994); – 5.68 in *P. densiflora*; 4.86 in *P. thunbergii*; – 3.05 in *P. koraiensis* (KIM & LEE 1995) and 3.42 as the overall mean of 16 pine species (GOVINDARAJU 1988). Using the private allele method (SLATKIN 1985) the

effective exchange of migrants between populations $Nm_{(S)}$ was three per generation. The values of F_{ST} and Nm differed among the five islands but always indicate a level of genetic flow of $Nm > 1$ except for the island of Gomera (Table 3). On the one hand genetic flow of $Nm > 1$, may be explained by air-disperse of pollen or seeds, and on the other hand, the relatively low gene flow level may reflect the history of the species and its often isolated populations due to site conditions.

Results of gene flow according to the Island model, *i.e.*, level of $Nm_{(W)}$ among Islands show a decreasing flow from the island of La Palma to Gran Canaria via Tenerife (4.65 to 3.13 to 2.53) (Table 3). Gene flow was not estimated for the Islands El Hierro and Gomera populations because of the very low sample size. Estimated gene flow using CROW & AKOI (1984) formula, *i.e.*, $Nm_{(C)}$ has also a decreasing tendency of gene flow, as said above, from the island of La Palma to Gran Canaria. Estimations of decreasing gene flow at larger spatial scales increased efficiency of selection and genetic differentiation in regional populations.

Estimation of Nm are obtained by indirect methods, therefore, estimated gene flow may differ from the real gene dispersion among populations (SLATKIN 1987) and populations may deviate from the Island model. SLATKIN & BARTON (1989) estimated Nm based on the frequencies of unique alleles found in only one local population; we found that dispersion of extensive gene flow among populations ranged from 0.0 to 11.95. In the three largest islands the value of $Nm_{(S)}$ differed from the value of $Nm_{(W)}$ and $Nm_{(C)}$; but the reduction in gene flow from La Palma to Gran Canaria via Tenerife was similar and was related to the geographic distance. Estimations of gene flow that are based on rare allele frequencies provide addition information, namely on how alleles disperse. The decrease of neutral alleles flow leads to genetic differentiation between populations, so that differentiation between populations of *P. canariensis* increases/decreases along a vector Gran Canaria – La Palma via Tenerife. The lower Nm values at Gomera could be explained by the lower effective population size, and in this case the increased genetic drift. The low gene flow and genetic differentiation at Gran Canaria could be explained by environmental factors.

SLATKIN (1993) suggested to test isolated population by the measuring of gene flow between pairs of populations. In the present study extensive gene flow $Nm > 1$ was found among *P. canariensis* populations (Table 1). Thus, since Nm depends on geographic distance we plotted pairwise Nm values against geographic distance with no significant results. However, when $Nm_{(W)}$ values and distance (km) within the pairs were plotted on a log-scale diagram a slight drop in the

gene flow with the increase of distance between pairs of islands was found. The sampled pairs could be divided into three distinct groups with three pairs in the first group and five pairs in the second one and two pairs in the third one. Differences in the genetic distances between the groups were low but they were significant. Mean genetic distance for islands group one was 0.0037 and for the second islands group 0.0100. Exchange of migrants within the first group which include the islands of La Palma, Gomera and El Hierro was relatively high with gene flow vectors: El Hierro – Gomera (8.33), El Hierro – La Palma (4.79) and La Palma – Gomera (3.95). Geographically these populations are not widely separated and a greater gene flow could reasonably be expected in this region in spite of the bioclimatic conditions that influence genetic differentiation of populations. The absence of an apparent pattern of isolation by distance in *P. canariensis* suggests that this species is far from equilibrium. Gene flow among the pairs of islands within the second and the third group averaged 2.68 and 3.01 with no specific direction or relations to distances between the islands.

The average estimated gene flow among population grouped according to elevation (Table 4) was between 2 and 4 migrants. However Nm values calculated according to WRIGHT (1978) show a decreasing differentiation (F_{ST}) with the increase in elevation, a similar tendency was obtained in *Pinus brutia* (KARA *et al.* 1997). The number of unique alleles (P_i) decreased with the increase in the height above sea level, we suggest that this tendency occur in wind-pollinated tree species because gene flow are estimated based on genetic variances among populations which is according to our data, related to height. Gene flow values within each of the six ecological groups (Table 4) range from $Nm_{(S)}$ 1.7 to 13.2, $Nm_{(W)}$ ranged from 2.5 to 8.7, and $Nm_{(C)}$ – from 0.63 to 3.86; the results show that within each of the these zones there is enough gene flow.

D (genetic distance) depends on mutation rates, Nm is a direct measure of gene exchange among populations and is independent from mutation rates (SLATKIN 1993). Consequently D is more appropriate for the estimation of divergence times between different species whereas Nm is more appropriate for the characterization of gene dispersal between populations within a single species (YANG & YEY 1995). Nevertheless, a time divergence between isolated populations may be estimated roughly according to equation: $t \gg D/2a$, where D is the genetic distance (NEI 1975) and a is the mutation rate. This equation may be used only approximately because of the tectonic activity and bioclimatic conditions on the islands which probably influenced the development of populations after their divergence

(FADY & CONKLE 1993). Divergence between the populations of *P. canariensis* on the Canary islands of La Palma, Tenerife, Gran Canaria started according to the formula 30.000–40.000 years ago.

To conclude, on the base of three different methods the estimated gene flow (Nm) of between two to three migrants per generation was confirmed. Such a gene flow is enough to prevent local genetic differentiation in populations, islands and ecological zones. The results calculated according to the three different methods had the similar tendency.

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