GENETIC DIVERSITY IN NATURAL POPULATIONS OF *ALNUS ACUMINATA* SSP. *ARGUTA* (SCHLECTENDAL) FURLOW IN COSTA RICA AND PANAMA.

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ABSTRACT

Alnus acuminata ssp *arguta* (Schlect.) FURLOW (*Betulaceae*) is a monoecious, nitrogen fixing pioneer tree species, that occurs throughout Latin America, from central Mexico to northern Panama. 17 natural populations (54 trees/population on average) were sampled from Costa Rica and Panama. Genetic variation was assessed at putative gene loci coding for 10 isoenzyme systems. Only 4 gene loci were polymorphic. Average polymorphism (16 %), number of alleles per polymorphic locus (2.0), allelic diversity (1.29) and average heterozygosity (4 % for all loci, and 22 % for polymorphic loci) were very low in comparison to other *Alnus* species and other tropical trees species. Cluster analysis revealed a pattern of genetic variation associated with geography for most populations. Genetic differentiation among populations, even within the same geographical region, was larger than reported previously for other *Alnus* species. Repeated bottlenecks due to its early successional behavior and its association to natural catastrophies, interspaced by periods of population expansion, are the main pattern shaping the population dynamics of this species. Low gene flow, due to partial geographical isolation among and within regions, may contribute to the relative large population differentiation (11 % overall gene differentiation among populations was detected, with over 5 % due to among populations within same geographical region and over 5.5 % among regions). *Idh–A* showed the largest genetic differentiation among populations and regions (15 %). The last glacial and interglacial events possibly played an important role for the re-establishment of migration and gene flow among the disjuncted regions of *Alnus* occurrence throughout Latin America.

Key words: *Alnus acuminata*, isozymes, genetic structure, Costa Rica, Panama

INTRODUCTION

Alnus acuminata ssp *arguta* (Schlectendal) FURLOW is a monoecious pioneer tree species which fixes nitrogen symbiotically with the actinomycete *Frankia* (RUSSO 1990). This wind-pollinated, fast growing tree occurs discontinuously throughout Latin America, from central Mexico to northern Panama (FURLOW 1979a; FURLOW 1979b). Taxonomic confusion still persists for the Latin American *Alnus* species (MURILLO *et al.* 1993). In an extensive taxonomical review of the *Alnus* genus in the American continent by FURLOW (1979a), states that all the Latin American species are very closely related, indicating relatively recent speciation. The author argues that the most primitive present segment of the complex is *A. acuminata* ssp *arguta* in southwestern Mexico, while *A. jorullensis* and the other subspecies of *A. acuminata* appear to have diverged primarily in response to differing climates at various altitudes. Hence, he stated that the area of best development and greatest variability of *Alnus acuminata* is southern Mexico and northern Central America, pointing out to this region as a possible site of origin. This tree species distributes disjunctly within Costa Rica and Panama between 1500 and near 3000 m in elevation (CATIE 1995), but occasionally from 1300 m (in Coronado, Irazú region). The species occurs naturally in different ecological regions, along the Central Volcanic to the Talamanca Mountain Ranges, conforming discontinous populations refuged above 1500 m in several volcanoes and mountains within these two countries (ALVAREZ 1956; CAMACHO & MURILLO 1986). In lower elevations it is restricted to streams and poorly drained areas. Some evidence of isolation by distance and a weak gene flow among geographic regions was found (MURILLO & ROCHA 1999).

Studies have been published on the morphological variation of *Alnus* in Costa Rica (MURILLO *et al.* 1993; VÍLCHEZ & MURILLO 1994; 1996) as well on Colombian *Alnus* populations (HERNÁNDEZ & RESTREPO 1995; RESTREPO-URIBE & BELLEFLEUR 1996). Its economic importance increased in the region, and more contributions on its management and ecology have recently been published (GRAU 1985; DEL VALLE &

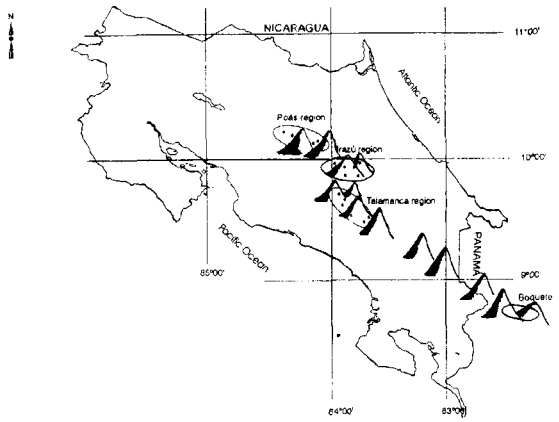

Figure 1. Natural populations of *Alnus acuminata* ssp. *Arguta* (Schlectendal) Furlow in Costa Rica and Panama. Circles show the regions of occurrence and dots the sampled populations.

GONZÁLEZ 1988; RUSSO 1990). In Costa Rica there are over 2000 ha planted and it is the second most important tree species for reforestation in the high elevations. As a result, some preliminary experiences from an ongoing breeding program in Costa Rica become also recently available (CORNELIUS *et al.* 1996). However, there is neither information on levels of genetic variation nor the population structure of this species in order to support a better sound reforestation program.

Populations from temperate tree species are often sampled throughout their entire range of distribution covering many thousands of kilometers, while sampling of tropical trees often involves populations separated by only a few kilometers (HALL *et al.* 1994). Results presented here cover a wide range of this common tree throughout its range in Costa Rica and Panama. The study contributes to a better understanding of the ecology and population genetics of *Alnus acuminata*. It may serve for guiding conservation and breeding programs in Central America.

MATERIAL AND METHODS

Studied populations

Materials was collected from randomly selected trees of 17 natural populations of *Alnus acuminata*. Most natural populations throughout the distribution of the tree species in Costa Rica and Panama were included in this genetic inventory. The populations were grouped into their four geographical regions of occurrence as shown in Figure 1 and Table 1. 31 to 63 (average 54) adult trees per population were randomly sampled (separated by no less than 50 to 100 meters). A total of 930 trees were investigated.

Electrophoretic methods

Horizontal starch gel electrophoresis was used to analyse the isozyme patterns in flower buds (both male and female), terminal buds and young terminal leaves. A total of twenty-two putative loci coding for ten isozyme systems were investigated for all sampled trees. The following enzyme systems were screened: aspartate aminotransferase (*Aat*) {2.6.1.1}, isocitrate dehydrogenase (*Idh*) {1.1.1.4.2}, leucine aminopeptidase (*Lap*) {3.4.11.1}, malate dehydrogenase (*Mdh*) {1.1.1.37}, menadione reductase (*Mnr*){1.6.99.2}, 6-phosphogluconate dehydrogenase (6-*Pgdh*) {1.1.1.44}, phosphoglucose isomerase (*Pgi*) {5.3.1.9}, phosphoglucomutase (*Pgm*){2.7.5.1}, shikimate dehydrogenase (*Skdh*){1.1.1.25} and alcohol dehydrogenase (*Adh*) {1.1.1.1}. Details of laboratory procedures are reported elsewhere (MURILLO & HATTEMER 1997).

Regular meiotic segregation was tested previously for the four polymorphic loci found in this study. Seedlings of single tree seed progenies derived from open pollination were used following the procedures of GILLET & HATTEMER (1989). The results confirmed the hypothesis of single loci with codominant alleles (MURILLO & HATTEMER 1997).

Measures of genetic diversity and differentiation

Allelic and genotypic structures were computed for each polymorphic locus and each population. All populations were regarded as effectively infinite in size and were weightened equally for the computation of differentiation measures. Deviations of genotypic structures from Hardy-Weinberg proportions and the heterogeneity of allelic frequency distributions across populations were tested for statistical significance using the log likelihood test (*G*-test) and Pearson's χ^2-test (GILLET 1994).

The following parameters of genetic variation within populations were computed: Percentage of polymorphic gene loci (PPL), average number of alleles per locus (A/L), expected heterozygosity H_e (="gene diversity", NEI 1973) or differentiation within an effectively infinite population d_T (GREGORIUS 1987)), observed heterozygosity H_o, allelic Diversity v (=effective number of alleles per locus), and hypothetical gametic diversity v_{gam} (GREGORIUS 1978). The values for A/L, H_e, H_o, and v were computed considering polymorphic loci only and considering all loci, i.e. including the monomorphic loci. The calculation of measures characterizing population differentiation is based on the four polymorphic loci only. Genetic distances d_0 (GREGORIUS 1984) were computed for all pairs of populations. Differentiation among populations was assessed using

Table 1. Sampled populations of *Alnus acuminata* ssp. *arguta* (Schlectendal) Furlow from Costa Rica and Panama.

Region/Population	Range of elevation	Latitude	Longitude	Approx. size of population N_i
Region Poas				
Zarcero	1700–1800	10° 14'	84° 22'	3 600
Bajos del Toro	1400–1500	10° 13'	84° 19'	12 375
Vara Blanca	1700–1800	10° 10'	84° 07'	3 780
Los Cartagos	2000–2100	10° 9'	84° 09'	19 500
Region Irazú -- Turrialaba				
Coronado ≤1400	1300–1400	9° 59'	84° 00'	2 250
Coronado ≥2000	2000–2500	9° 58'	83° 56'	15 000
Llano Grande	1700–1900	9° 55'	83° 56'	6 000
Irazú	2900	9° 57'	83° 49'	300
Pacayas	1700–1800	9° 55'	83° 48'	6 750
Turrialba	2500–2600	9° 58'	83° 47'	300
Region Talamanca				
El Empalme	2100–2400	9° 45'	83° 57'	3 000
Cañón	2300–2400	9° 42'	83° 54'	3 000
Copey	1700–1900	9° 39'	83° 55'	9 000
San Gerardo	2000–2200	9° 33'	83° 49'	4 500
Siberia	2700–2800	9° 33'	83° 41'	300
División	2000–2200	9° 31'	83° 42'	11 250
Region Boquete, Panama				
17. Boquete	1400–1600	8° 45'	82° 20'	12 000
Range	1300–1900	8° 45' – 10° 14'	82° 20' – 84° 22'	

N_j = Estimated population size = (area (ha) from population j) × (average tree density (N/ha)) (KAPELLE *et al.* 1995, FLORES 1995; personal communication).

the concept of GREGORIUS and ROBERDS (1986), which is based on genetic distances d_0 of populations to their respective complements. We also partitioned the total gene diversity according to NEI (1973), into a component due to differentiations among regions (G_{RT}), a component due to differentiation among populations within regions (G_{SR}), and a component due to variation within populations (G_{SP}). The contribution of each population to the total gene diversity was computed according to FINKELDEY & MURILLO (1999). The contribution of a population due to its own diversity is simply the within-population gene diversity divided by the number of investigated populations n. The contribution of a population due to its differentiation is the mean of Nei's minimum genetic distances to all populations also divided by n. The total contribution of a population to gene diversity is the sum of both components. The following software packages were used for the computation of the diversity and differentiation measures: GSED (GILLET 1994) and BIOSYS (SWOFFORD & SELANDER 1981).

RESULTS

Genetic variation within populations

Out of the 22 putative loci in all populations surveyed, only four were polymorphic (*Pgm–A*, *Pgi–B*, *Idh–A* and *Mnr–A*). No variation was observed at the remaining eighteen putative loci. Allelic frequency distributions for the 4 polymorphic gene loci are listed in Table 2.

Out of 68 tests performed, deviations from Hardy-Weinberg proportions at the 5 % level of significance were found only for population El Empalme at the *Mnr–A* locus (G-and χ^2 test), for population Cañón at the *Pgi–B* locus (G-test only), and for population Turrialba at the *Pgi–B* locus (χ^2 test only).

All parameters indicate very low genetic diversity in

Table 2. Frequency distributions of alleles at all polymorphic gene loci in populations of *Alnus acuminata* from Costa Rica and Panama.

Region	Population	No of trees	*Mnr-A*		*Idh-A*		*Pgm-A*			*Pgi-B*		
			A_1	A_2	A_1	A_2	A_1	A_2	A_3	B_1	A_2	B_3
Poas	Zarcero	62	1.00	0.00	0.83	0.17	0.20	0.00	0.80	0.09	0.75	0.16
	Bajos Toro	61	1.00	0.00	0.84	0.16	0.11	0.06	0.83	0.03	0.93	0.04
	Vara Blanca	61	0.99	0.01	0.57	0.43	0.21	0.00	0.79	0.16	0.72	0.12
	Cartagos	61	0.98	0.02	0.58	0.42	0.06	0.00	0.94	0.02	0.75	0.23
Irazú	Coronado ≤1400	43	0.95	0.05	0.51	0.49	0.13	0.00	0.87	0.00	0.82	0.18
	Coronado ≥2000	60	0.97	0.03	0.60	0.40	0.01	0.00	0.99	0.00	0.85	0.15
	Llano Grande	60	1.00	0.00	0.52	0.48	0.04	0.00	0.96	0.00	0.79	0.21
	Irazú	48	1.00	0.00	0.60	0.40	0.02	0.00	0.98	0.00	0.99	0.01
	Pacayas	62	1.00	0.00	0.65	0.35	0.03	0.00	0.97	0.00	0.88	0.12
	Turrialba	50	1.00	0.00	0.25	0.75	0.02	0.00	0.98	0.00	0.85	0.15
Talamanca	El Empalme	63	0.98	0.02	0.82	0.18	0.06	0.00	0.94	0.00	0.82	0.18
	Cañón	61	0.97	0.03	0.78	0.22	0.05	0.00	0.95	0.00	0.75	0.25
	Copey	63	0.99	0.01	0.93	0.07	0.18	0.00	0.82	0.00	0.66	0.34
	San Gerardo	48	0.99	0.01	0.74	0.26	0.14	0.00	0.86	0.00	0.63	0.37
	Siberia	31	0.98	0.02	0.91	0.09	0.12	0.00	0.88	0.00	0.66	0.34
	División	61	0.98	0.02	0.93	0.07	0.11	0.00	0.89	0.00	0.82	0.18
Boquete	Boquete	35	1.00	0.00	0.76	0.24	0.34	0.04	0.62	0.00	0.54	0.45
Range	lowest value	31	0.95	0.00	0.25	0.07	0.01	0.00	0.62	0.00	0.54	0.01
	highest value	64	1.00	0.05	0.93	0.75	0.34	0.06	0.99	0.17	0.99	0.45
Weighted average			0.987	0.013	0.697	0.303	0.105	0.005	0.890	0.020	0.785	0.195

all populations (Table 3). Population Irazú is particularly poor in genetic variation. Comparatively high variation was observed in populations Vara Blanca, Los Cartagos, Bajos del Toro and Boquete. The first three populations and Boquete are located at the two extremes of the natural distribution range of this tree species in this part of Central America (Figure 1).

The average level of heterozygosity is very low with a mean value of only $H_e = 0.04$ if all 22 gene loci are considered.

The expected heterozygosity at the polymorphic gene loci (Table 3) is largest in Boquete ($H_e = 0.34$) as lowest in Irazú ($H_e = 0.13$). Population Turrialba contributes the largest proportion to the overall gene pool genetic differentiation ($D_{ST(j)}$ within populations, followed by population Boquete (Table 4).

We tested the hypothesis that small populations are particularly depauperate of genetic variation by plotting the expected heterozygosity (gene diversity) against population size (Figure 2). No clear trend is obvious, i.e. small actual census population sizes are not closely associated with low gene diversity. The correlations between gene diversity and population size (N_j) showed values almost equal to zero (Figure 2).

Variation among populations

Allelic frequency distributions show considerable heterogeneity among populations at the three loci *Pgi–B*, *Pgm–A*, and *Idh–A*. The allele *Pgi–*B_1 was observed in all populations of the Poás region, but not in any other population out of this region (Table 2). The allele A_1 has a frequency of at least 95 % in all populations. Thus, differentiation is low at this locus showing a typical minor polymorphism. Populations Turrialba and Boquete are most strongly differentiated from all other populations as indicated by the highest value of D_j for these populations (Table 4). The hierarchical partitioning of gene diversity shows that most variation is within populations (89.4 %; cf. "gene pool" G_{SP} in Table 5). The gene diversity due to differentiation among populations is 10.6 % and approximately equally distributed between the components due to differentia-

Table 3. Measures of genetic variation within populations of *Alnus acuminata* in Costa Rica and Panama.

Population	Percent of polymorpic loci	4 polymorphic loci				All loci				$\upsilon_{gametic}$
		A/L	H_e	H_o	Diversity υ	*A/L*	H_e	H_e	Diversity υ	
Zarcero	80	2.00	0.25	0.24	1.32	0.8	0.10	0.043	1.13	3.31
Bajos Toro	90	2.25	0.17	0.15	1.21	0.9	0.07	0.026	1.08	2.26
Vara Blanca	90	2.25	0.32	0.34	1.47	0.9	0.13	0.062	1.19	5.40
Cartagos	90	2.25	0.26	0.22	1.34	0.9	0.10	0.040	1.14	3.71
Coronado ≤1400	80	2.00	0.28	0.24	1.38	0.8	0.11	0.044	1.15	3.99
Coronado ≥2000	80	2.00	0.29	0.19	1.25	0.8	0.09	0.035	1.10	2.76
Llano Grande	70	1.75	0.23	0.22	1.29	0.7	0.09	0.040	1.12	3.23
Irazú	70	1.75	0.13	0.14	1.16	0.7	0.05	0.025	1.06	2.05
Pacayas	70	1.75	0.18	0.18	1.22	0.7	0.07	0.032	1.09	2.48
Turrialba	70	1.75	0.17	0.13	1.20	0.7	0.07	0.024	1.08	2.23
El Empalme	80	2.00	0.16	0.20	1.23	0.8	0.06	0.035	1.09	2.36
Cañón	80	2.00	0.22	0.23	1.27	0.8	0.09	0.043	1.11	2.82
Copey	80	2.00	0.22	0.22	1.29	0.8	0.09	0.040	1.12	3.03
San Gerardo	80	2.00	0.28	0.29	1.38	0.8	0.11	0.053	1.15	4.04
Siberia	80	2.00	0.17	0.26	1.28	0.8	0.07	0.045	1.11	2.88
División	80	2.25	0.14	0.15	1.19	0.9	0.06	0.027	1.07	2.05
Boquete	80	2.00	0.34	0.36	1.52	0.8	0.14	0.065	1.21	6.30

Table 4. Genetic differentiation among populations (D_j and δ; GREGORIUS & ROBERDS 1986), partitioning of the overall contribution of single populations ($H_{T(j)}$) to total gene diversity due to their gene diversity ($H_{S(j)}$) and due to their differentiation from other populations ($D_{ST(j)}$), and ranking of the populations in the order of their contribution to total gene divesrity (FINKELDAY & MURILLO 1999). All values are for the gene poool (4 polymorphic loci).

Population	D_j	$D_{ST}(j)$	$H_s(j)$	$H_T(j)=D_{ST}(j)+H_s(j)$	Rank
Zarcero	0.071	0.0012	0.0148	0.0160	6
Bajos Toro	0.098	0.0015	0.0102	0.0118	14
Vara Blanca	0.104	0.0014	0.0189	0.0203	2
Cartagos	0.070	0.0010	0.0151	0.0161	5
Coronado ≤1400	0.080	0.0013	0.0164	0.0178	3
Coronado ≥2000	0.094	0.0011	0.0120	0.0131	12
Llano Grande	0.081	0.0013	0.0134	0.0147	8
Irazú	0.113	0.0017	0.0079	0.0096	17
Pacayas	0.074	0.0010	0.0107	0.0117	15
Turrialba	0.166	0.0039	0.0098	0.0137	11
El Empalme	0.059	0.0011	0.0109	0.0120	13
Cañón	0.045	0.0010	0.0128	0.0138	10
Copey	0.112	0.0019	0.0131	0.0150	7
San Gerardo	0.051	0.0012	0.0163	0.0175	4
Siberia	0.083	0.0017	0.0127	0.0144	9
División	0.081	0.0016	0.0097	0.0113	16
Boquete	0.153	0.0027	0.0200	0.0227	1
Mean (δ)	0.090				
Total		0.0266	0.2247	0.2514	

tion among populations within regions and due to differentiation among regions. A considerable amount of gene diversity among regions was observed at the three polymorphic loci *Pgi–B*, *Pgm–A*, and *Idh–A* (Table 5).

Population Boquete contributes most to the total gene diversity followed by population Vara Blanca (Table 4). Turrialba is most strongly differentiated from

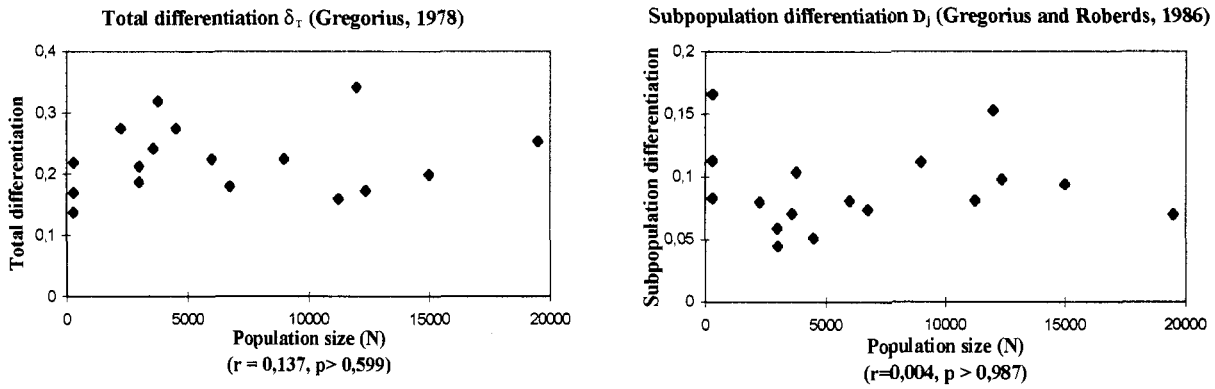

Figure 2. Relationship between population size and genetic differentiation in populations.

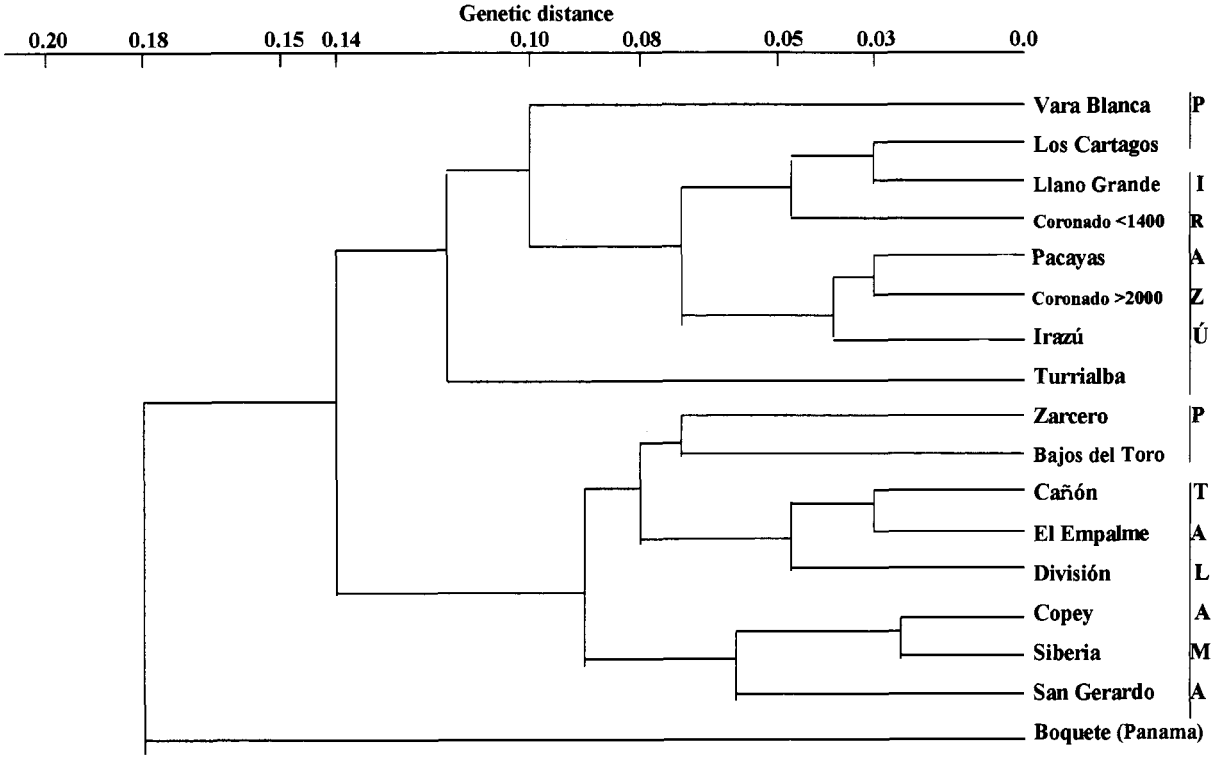

Figure 3. UPGMA dendrogram for the natural populations of *Alnus acuminata* ssp. *Arguta* in Costa Rica and Panama.

the other populations as indicated by its highest values both for D_j and $D_{ST}(j)$. However, it is a population of low genetic diversity since $H_S(j)=H_e/n=0.0098$. Thus, it ranks only as 11th in its contribution to total gene diversity. The contribution of population Irazú to the total gene diversity is only 0.0096 and thus only 42.3 % of the value for population Boquete. Thus, considerable differences exist with regard to the contribution of populations to the total gene diversity although the partitioning of the genetic diversity resulted in 89.4 % of the total gene diversity residing within populations. Genetic drift affects mainly small populations and may lead to stronger differentiation of small populations.

However, no such effect is evident if estimated population sizes are plotted against the subpopulation differentiation D_j (Figure 2). The dendrogram (Figure 3) reflects the geographical distribution of the investigated populations into different regions surprisingly well considering the small number of gene loci and their low variation. One main cluster comprises the populations of region Irazú and the populations Vara Blanca and Los Cartagos of the Poás region. A second main cluster comprises the Talamanca region and the remaining two populations of region Poás. Population Boquete is clearly separated from both of these clusters.

DISCUSSION

Genetic diversity

In spite of extensive sampling of the species throughout its range in Costa Rica and Panama, relatively little genetic variability was detected. The average percentage of polymorphic gene loci (PPL) of 16.3 % is low in comparison to PPL from 27.6 to 43.6 % reported for other tropical tree species sharing similar ecological conditions (HAMRICK & LOVELESS 1986; HAMRICK *et al.* 1992). A mean value of 1.27 alleles per locus is very low if compared to other *Alnus* species from Canada reported on average of 1.8 to 2.3 alleles per locus (BOUSQUET & LALONDE 1991). *Alnus glutinosa* of Germany is also reported as more variable (LINARES-BENSIMÓN 1984; STEINER, person. comm.). Even if only the polymorphic loci are considered, the mean number of alleles per locus is 2.0 in *A. acuminata,* which does not fall either into the range between 2.22 and 2.78 reported for the *Alnus* species from Canada by BOUSQUET *et al.* (1987a, b, c; 1988; 1990), nor into the values (2.17) reported for a group of 16 uncommon tropical tree species (HAMRICK & MURAWSKI 1991). However, these estimates may be biased downwards owing to the exclusion of polymorphic zones with uninterpretable banding patterns (MURILLO & HATTEMER 1997). Average heterozygosity was much lower for *A. acuminata* (H_e = 0.04) than reported for other *Alnus* species, which range between H_e = 0.107 in *A. crispa* and H_e = 0.165 in *A. rugosa* (BOUSQUET *et al.* 1987a, b, c; 1988; 1990). The "expected heterozygosity" of *A. acuminata* is low (H_e = 0.22) even if only the four polymorphic loci are considered. HAMRICK *et al.* (1992) report corresponding values of between 0.248 and 0.328 for other species that share its list of ecological characteristics of a short-lived perennial, large natural distribution, wind-pollinated temperate-tropical genus of early successional status.

It is unlikely that sample size had a major effect on the observed allele frequencies (EL-KASSABY 1991), since an average of 54 trees per population was randomly sampled after extensive field work. However, variation patterns at single gene loci had a strong impact on gene pool values due to the low number of polymorphic gene loci. For example, the strong genetic differentiation of population Turrialba is mainly explained by a single gene locus (*Idh–A*), as detected in the parameter D_j (Table 4) and reflected in dendrogram (Figure 3).

Repeated bottlenecks due to the early successional behavior and the association to natural catastrophes in relatively short intervals, interspaced by periods of population expansion and recolonization, are likely to be the dominant patterns shaping the population dynamics of this species. *A. acuminata* is a pioneer species that requires large open and disturbed areas in order to be able to colonize and expand. Its symbiosis with *Frankia* spp allows it to survive and compete even in the complete absence of soil (VÍLCHEZ & MURILLO 1994). A dynamics of frequent extinction of small subpopulations and ephemeral recolonization of newly suitable patches of habitat prevails within regions. Some of these patches are presently large (Table 1), favored either by anthropogenic disturbance or by recent natural catastrophes. The two populations with the lowest genetic diversity (Table 3) are the ones which suffered a recent large volcanic eruption-period (Irazú in 1963–1964) and an over 7.5 Richter scale's earthquake (División in 1986). Since these two populations are not apparently isolated from other populations in their regions (Irazú and Talamanca regions respectively), their genetic structure support the hypothesis of recent bottleneck. Thus, the population dynamics of this tree species is highly determined by the magnitude and frequency of such events, which are the typical pattern along the volcanic mountain chains of Central America and the Andes. The very low genetic variation of the other populations of the Irazú region may be explained by natural catastrophes caused during the last eruption-period of the Irazú volcano (1963–1964). Populations Coronado above 2000 m, and possibly Llano Grande, suffered a severe size reduction and are the result of a very recent recolonization process originating from very few scattered remaining adult trees, resulting in presently low population sizes and a transient bottleneck. Such transient behavior influences not only population size, but also heterozygosity, and number of alleles per locus owing to stochastic factors (MARUYAMA & FUERST 1985a; MARUYAMA & FUERST 1985b; MOSSELER 1995). Levels of genetic diversity critically depend on the duration of the expansion and reduction phases and also on the mutation rate. The rate of growth of the population following a bottleneck will define the reduction in gene diversity; otherwise, reductions in heterozygosity due to inbreeding will result in further losses of genetic diversity (MARUYAMA & FUERST 1985a; MOSSELER 1995). The low levels of polymorphism and low numbers of alleles in the populations of the Irazú region are probably a better indicator of a recent and severe bottleneck than levels of heterozygosity or allelic diversity (LEBERG 1992). Under the conditions of small population size, genetic drift could lead to a rapid loss of alleles. Particularly, rare alleles are lost rapidly (MARUYAMA & FUERST 1985b). However, the high reproductive capability (VÍLCHEZ & MURILLO 1994, 1996), discrete overlapping generations, high colony density (KAPPELLE *et al.* 1995;

FLORES 1995) and colonizing vigor of *A. acuminata* may have counteracted population extinction. The amount of genetic diversity within a population is the result of its evolutionary past. Low population sizes and bottlenecks in the past reduce genetic variation. Current population sizes are poor indicators of past sizes for species capable of rapid population expansion e.g. after catastrophic events. Small population sizes in the recent or more distant past resulting in genetic drift and low genetic diversity cannot be ruled even for currently large census populations of *A. acuminata*. In *A. acuminata* populations in Costa Rica, trees reach reproductive stage after 6–7th year-old and above 86 % of them flower and seed abundantly each year (VÍLCHEZ & MURILLO 1994, 1996). Thus, effective population size can be estimated in about 80 % or more from census population in this pioneer tree species. Low levels of genetic diversity were also reported from *Pinus merkusii* in Thailand. This tropical pine is also capable of rapid population expansion after large-scale forest disturbance and even large populations may be founded by only a few trees (CHANGTRAGOON & FINKELDEY 1995).

Another possible reason for low genetic diversity is the disjunct distribution area of *A. acuminata* populations in Costa Rica and Panama and the more distant evolutionary past with the rest of the latinamerican distribution (FURLOW 1979b). Geographical isolation occurred in this region during the interglacial times. The dynamics of repeated glacial and interglacial events during the last million of years permitted *Alnus* migration through the Central American filter (RICH & RICH 1983; GÓMEZ 1986), as well as the loss and reestablishment of the contact between populations. Geographical isolation within Costa Rica and Panama occurred, since the populations refugia were on peaks (above 1500 m elevation) of separated volcanoes and mountains. During the last glacial period in this region (approximately 11300–9600 years ago), the actual timber line was around 600 to 1000 m below its actual position (ALVARADO 1994). Therefore, at that time, *A. acuminata* presented a continuous natural distribution within Costa Rica and Panama. Similarly, the re-establishment of contact with the other disjunct populations from southern Mexico and the northern region of Central America, or even with the Andean populations, likely depended on the extent of the glacial events.

Spatial patterns of genetic variation

We found considerable and significant differences in allele frequencies at the three polymorphic gene loci *Pgi–B*, *Pgm–A*, and *Idh–A* although the partitioning of genetic variation showed that almost 90 % of the total variation is within populations (Table 5). Genetic differentiation among populations of Canadian populations of *Alnus crispa* and *A. rugosa* (BOUSQUET *et al.* 1987a,b,c; BOUSQUET and LALONDE 1991) and of *A. glutinosa* (PRAT *et al.* 1992) in France is lower although populations were sampled from a wider range. The maximum geographical distance between our populations is only 280 km.

Wind is the principal vector for the pollen and seeds of *A. acuminata*. The average genetic differentiation of outcrossed, wind-pollinated, woody species is G_{ST} = 0.077. The correspondig values for woody species with seed dispersal by wind and for all tropical tree species are G_{ST}= 0.076 (HAMRICK *et al.* 1992) and G_{ST} = 0.096 (LOVELESS 1992), respectively. The total proportion of the gene diversity due to differentiation among populations of *A. acuminata* is $G_{SR} + G_{RT} = G_{ST} = 0.104$ (Table 5) and thus above the average of species with comparable mechanisms of pollen- and seed disperal, and slightly above the average value for tropical tree species.

Considerable amounts of genetic differentiation were found at the three most variable gene loci among populations within regions and among regions as well (G_{SR} and G_{RT}; Table 5). The rare allele $Pgi\text{–}B_1$ was confined in its distribution solely to the Poás region with quite a large frequency (7.5 % in average). Population Boquete in Panama, at the southern extreme of the studied range, shows quite different allele frequencies in almost all cases with respect to all other geographical regions (Table 2). These results suggest that there is a very low exchange of genes among regions and a clear isolation by distance pattern.

Strong differentiation was also observed at the

Table 5. Partitioning of the total gene diversity of *Alnus acuminata* into relative components due to variation within populations (G_{SP}), components due to variation among populations within regions (G_{SR}), and components to variation among regions (G_{RT}) for 4 gene loci and the gen pool (NEI 1973).

	Pgi–B	*Pgm–A*	*Idh–A*	*Mnr–A*	Gene pool
G_{SP}	0.924	0.920	0.851	0.985	0.894
G_{SR}	0.039	0.029	0.074	0.017	0.051
G_{RT}	0.037	0.051	0.075	-0.002	0.055

Idh–A locus among populations of the Irazú region. Population Turrialba is separated by a distance of only approximately 10 *km* to populations Irazú and Pacayas, but it is clearly differentiated from both populations at the *Idh–A* gene locus. The strong genetic differentiation within a comparatively small area also suggests only limited gene flow in some areas in this region. Similarly in Poás region, there was detected a strong differentiation between populations Zarcero and Bajos del Toro versus populations Vara Blanca and Los Cartagos (Table 2). Only in the last two populations was observed allele $Mnr–A_2$ and allele $Pgm–A_2$ was observed only in Bajos del Toro with quite a large frequency (6 %). Thus, there were detected gene flow limitations among populations within this region as well. In Irazú region was observed a pattern of variation associated to populations Coronado £ 1400 and Coronado > 2000 with respect to the other four populations studied in this region. Only in these two populations (at western within region) was detected allele $Mnr–A_2$ and a clear geographical trend west-east, with lowest genetic variation in east direction (Pacayas, Irazú and Turrialba, Table 5). These results support the hypothesis of some degree of geographical isolation within regions as well. Another possible explanation for the comparatively strong differentiation among populations is selection. The investigated populations experience very different environmental conditions due to an altitudinal range of more than 1500 metres, different temperature and other climatic factors, and different edaphic conditions. Deep and rich volcanic soils (andosols) dominate in the Irazú and Poás region contrasting to shallow, infertile and poorly drained inceptisols in the Talamanca region (VÁZQUEZ-MORERA 1983). Further experimental investigations are needed in order to assess the role of selection in different environments and limited gene flow for the maintenance of genetic differentiation among populations.

The partitioning of genetic diversity showed that most variation is harboured within populations (Table 5). This seems to imply the equivalence of populations with regard to their suitability as genetic resources. However, isozymes are poor indicators for adaptive differentiation among populations (FINKELDEY 1994a, pp. 94), and the amount of genetic differentiation at isozyme gene loci in *A. acuminata* is above the average reported from species with similar life history characteristics. Furthermore, contributions of single populations to the total gene diversity differ considerably (Table 4). We suggest that the variation among contributions of single populations to total gene diversity may serve as an indicator for the different suitability of populations as genetic resources. Our results show strong differences among populations with regard to their contribution to total gene diversity and point towards populations contributing most to the total gene diversity (Table 4).

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