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Selecting populations for gene conservation purposes in forestry: a study case with *Alnus acuminata* in Costa Rica and Panama

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Abstract

In forest gene conservation programs there is a continuous discussion on criteria for population elections. Before actions are initiated, it is necessary to define what is a gene resource and which of the particular populations (entries) deserve highest priority. Most gene conservation programs are oriented to 1) yield potential: genetic potential for expressions of desirable phenotypic characters; (2) genetic adaptability: the ability of populations to survive and reproduce even in a changed environment; (3) conservation of as much variation as possible. This work presents the study case of *Alnus acuminata* in Costa Rica and Panama and an extensive discussion on different criteria for population selection. Variables evenness deviation, proposed in this study $\{ed = \sum | p_i - \bar{p} | /[2(n-1)/n]\}$ and genetic diversity $[v = (\sum_{i=1}^{n} p_i^2)^{-1}]$, both as culling levels, showed the best criteria for populations selection. At least there was found one good candidate population in each of the three most important geographical region (breeding region), suitable for gene conservation purposes: Vara Blanca (Region Poás II), San Gerardo (Region Talamanca) and Boquete (Region Panama). Possibilities of *in situ* gene conservation in natural protected areas are also discussed.

Key words: gene conservation, conservation genetics, population genetics, *Alnus acuminata*, Costa Rica, Panama, isozymes.

Resumen

Selección de poblaciones para conservación genética forestal: estudio de caso con *Alnus acuminata* de Costa Rica y Panamá

En los programas de conservación genética forestal existe una discusión permanente sobre los criterios de elección de poblaciones. Antes de iniciar acciones, es necesario que se defina que és un recurso genético y cuáles poblaciones en particular (accesos) merecen la más alta prioridad. La mayoría de los programas de conservación se orientan hacia 1) potencial de rendimiento: poblaciones con un potencial genético alto para la expresión de caracteres deseables; 2) adaptabilidad genética: la habilidad de las poblaciones para sobrevivir y reproducirse aún en condiciones de un ambiente cambiante; 3) conservación de la mayor variabilidad posible. En este trabajo se presenta el estudio de caso de *Alnus acuminata* en Costa Rica y Panamá, así como una discusión en extenso sobre diferentes criterios para la elección de poblaciones. La variable desviación de igualdad, propuesta en este estudio $\{ed = \sum | p_i - \bar{p} | /[2(n-1)/n]\}$, así como la variable diversidad genética $[v = (\sum_{i=1}^{n} p_i^2)^{-1}]$, ambas utilizadas como niveles de eliminación, mostraron los mejores criterios para la elección de poblaciones. Se detectó al menos una población candidata en cada una de las tres regiones geográficas más importantes (regiones de mejoramiento), apropiada para los propósitos de conservación genética: Vara Blanca (Región Poás II), San Gerardo (Región Talamanca) y Boquete (Región Panamá). Las posibilidades de conservación genética *in situ* en áreas naturales protegidas es también incluida en la discusión.

Palabras clave: conservación de genes, conservación genética, genética de poblaciones, *Alnus acuminata,* Costa Rica, Panamá, isoenzimas.

Introduction

There is a wide agreement among forest geneticists and the informed public that not only species should be conserved but also conserving genetic variation is a necessary element of any gene conservation program (Bund-Länder-Arbeitsgruppe «Erhaltung forstlicher Genressourcen der Bundesrepublik Deutschland», 1996). Not only is variation needed to ensure the present adaptability of species, but it is needed for the continued evolution of the species (Namkoong *et al.*,

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1996). However, there is a broad disagreement on the methods of gene conservation. Questions persist, for example, as to the most effective sample sizes, *in situ* vs. *ex situ* methods, and natural vs. artificial regeneration (Riggs, 1990; Brown and Hardner, 2000; Müller-Starck, 1995; Turok and Hattemer, 1995; Boshier and Young, 2000; Frankham *et al*., 2002). But today, most authors agree that dynamic conservation is generally preferable for forest trees, since there would be a larger adaptive change of a dynamically conserved genetic resource in response to environmental change, which may allow evolution to continue (Finkeldey and Gregorius, 1994; Hattemer, 1995; Turok and Hattemer, 1995).

In tropical countries has occurred a dramatic reduction of forest coverture in the last decades, promoting isolated patches of forest. These forest relicts prevent mating contact among individuals from different populations and therefore, promote severe reductions in migration and gene flow (Hattemer, 1996; Namkoong *et al.*, 1996; Frankham *et al*., 2002, chapter 13, pp. 309-334). Costa Rica is not the exception and the large deforestation rates in the last 40 years caused the elimination of forest from a 39% of the territory. However, the development of a national park system in the last 30 years, compressing today more than 25% of the country, allowed *in situ* preservation of almost all representative ecosystems (Vargas, 1994). *Alnus acuminata* ssp *arguta* (Schlectendal) Furlow is a monoecious, nitrogen fixer and pioneer tree species that occurs discontinously from central Mexico to northern Panama (Furlow, 1979a,b). This wind pollinated tree species distributes disjunctly within Costa Rica and Panama between 1,500 and near 3,000 m in elevation (CATIE, 1995). It occurs naturally in different ecological regions along the Central Volcanic to the Talamanca Mountain Ranges, conforming discontinous populations refuged above 1,500 m in several volcanoes and mountains within these two countries (Murillo *et al.*, 1993). In lower elevations it is restricted to streams and poorly drained areas (CATIE, 1995).

Before actions being initiated, it is necessary to define what is a gene resource and which of the particular populations (entries) deserve highest priority (declaration of a gene resource). A gene resource has been defined as a biological material that must either be known for or be expected to contain either some specific genetic information, or extensively variable genetic information (Hattemer, 1995). The spatial structure of patterns of genetic variation is crucial for the choice of genetic resources. Those populations with a low genetic differentiation with respect to the pool of all other eligible populations indicate that the candidate well represents the species (Gregorius and Roberds, 1986). At the same time, those populations at the edges of the distribution may differ strongly with respect to the rest of populations, which could indicate local adaptation (Gregorius and Bergmann, 1995). Therefore, has been proposed to preserve at least one viable population in each of the distribution patches within its natural range, as well as at least from each ecological distinct area (Finkeldey and Hattemer, 1993; Turok and Hattemer, 1995). The choice among candidate entries should be based on genetic diversity and multiplicity as the primary criteria (Hattemer, 1995). Besides, the measure of adaptive potential ($M\alpha$) should be based on the effective number of genetic variants with frequency above 2 to 3% (Finkeldey and Gregorius, 1994), or above 5% (Brown and Hardner, 2000). In the same direction has been discussed that, since alleles at frequency 0.01 or above 0.99 have little effect on additive genetic variance and hence, little immediately effect on adaptability, there is no need to save all alleles at all loci (Eriksson *et al.*, 1995; Frankham *et al*., 2002, chapter 14, pp. 336-357). Besides, most traits of significance are controlled by many gene loci and each locus has effects that may control the expression of many traits. Thus, the effect of one allele at one locus can often be substituted for by any one of several alleles at that or other loci which act in the same or several other metabolic pathways. Therefore, even though there are legitimate concerns for the role of rare alleles in long-term evolution, the resources could be managed for those concerns without having to create ever larger collections and reserves to store unique alleles (Eriksson *et al.*, 1995).

In terms of conservation objectives there are three major recognized (Ziehe *et al.,* 1989): (1) Yield potential: the genetic potential for expressions of desirable phenotypic characters. (2) Genetic adaptability: the ability of populations to survive and reproduce even in a changed environment. (3) Conservation of as much variation as possible. More specifically, Eriksson *et al.* (1995) mention that an important objective is the creation of good conditions for future evolution. That means the capture of genes at frequencies > 0.01 and most of the existing adaptations that may occur in different populations, which is most easily accomplished by developing a collection of metapopulations within the species.

Most of the opinions are in favor of the neutrality of the molecular markers and that they mainly reflect

long-term evolutionary history. While many metric traits are associated with adaptation and as a consequence reflect recent selection events (Eriksson *et al.*, 1995). In the other hand, there is more and more evidence that support an adaptational effect reflected in isozymes (Finkeldey and Gregorius, 1994; Gregorius and Bergmann, 1995). Therefore has been argued, that before a conservation sampling program is put into effect, it is important to investigate population structures for both types of traits. The Multiple Population Breeding System developed by Namkoong (1976) as well as conservation stands (Finkeldey and Hattemer, 1993) provide reliable *ex situ* methods in the context of dynamic conservation.

Based on the discussed arguments, here is developed a selection method for choosing population(s) for a gene conservation program, with *Alnus acuminata* in its natural distribution within Costa Rica and Panama.

Methodology

As a methodological study case, it was developed here the experience with *Alnus acuminata* native population's analysis, with samples from all its natural distribution in Costa Rica and Panama. The sampling procedures gathered material from more than 900 individual trees, within 17 natural populations. Distribution of the natural populations in five geographical regions within these two countries is shown in table 4.

Allozyme data

The 17 sampled populations represent geographically and ecologically the distinct natural regions of occurrence of *Alnus acuminata* ssp *arguta* in Costa Rica and Panama (CATIE, 1995; Murillo *et al.*, 1993). The different single populations are distributed along 5 natural regions of populations (Murillo, 1997). Each population was represented by a random sample of 31 to 63 adult trees (with an average of 52 trees) with a minimum of 50 m apart. Different materials from each tree for variation in 10 isozymes were electrophoretically assayed, but latter analyses were only performed on the four polymorphic and with clear resolution isozymes determined. Details about the tissue preparation, electrophoretic conditions, and enzymes scored were published elsewhere (Murillo and Hattemer, 1997).

Measures of genetic variation

Population genetic analyses using the «Genetic Structures from Electrophoresis Data» (GSED) software (Gillet, 1994) were performed. The genetic variation within populations and regions were determined (Murillo and Finkeldey, 2000), based on the following established parameters: diversity [$\nu = (\sum_{i=1}^{n} p_i^2)^{-1}$] of the gene pool and of the multilocus gametes (Gregorius, 1978, 1987); evenness (*e*) on each polymorphic allel (Gregorius, 1990); total population differentiation [$\delta_T = \frac{N}{N-1}\ (1 - \sum_{i=1}^{n} p_i^2) = \frac{N}{N-1}\ (1 - \frac{1}{\nu}\ ]$) (Gregorius, 1987); observed heterocigosity (H_o) and average degree of heterozygosity (H_G in Gregorius, 1978). The interpopulation variation was expressed by the subpopulation differentiation D_j (Gregorius and Roberds, 1986; Gregorius, 1988).

Evenness deviation

A new parameter, termed in this work as «evenness deviation» was determined. It is based on the simple sum of allele frequency deviations from a perfect evenness (equal allele distribution). Therefore, the absolute evenness deviation for a given gene locus (l) = $\sum | p_i - \tilde{p} |$, where $\tilde{p} = 0.50$ (for 2 alleles observed), $\tilde{p} = 0.33$ (for 3 alleles observed), $\tilde{p} = 0.25$ (for 4 alleles observed) and so on. But for comparison purposes is necessary to use a relative value, then the relative evenness deviation = 1/(max deviation)$\sum | p_i - \tilde{p} |$, where the maximum deviation increases with the number of alleles considered and can be estimated empirically from a hypothetical extreme deviated values in an allele frequency distribution as follows: for 2 alleles ($A_1 = 0.9999$ and $A_2 = 0.0001$), for 3 alleles ($A_1 = 0.9999$, $A_2 = 0.00005$ and $A_3 = 0.00005$), for 4 alleles ($A_1 = 0.9999$, $A_2 = 0.00005$, $A_3 = 0.00003$ and $A_4 = 0.00002$) and so on. Thus, the maximal evenness deviation for 2 alleles would be 1.0, for 3 alleles 1.333, for 4 alleles 1.5 and so on. This maximal evenness deviation can be also obtained through the relation 2(n – 1)/n (Gregorius, personal communication), where n is the number of alleles. Thus, evenness deviation = $\sum | p_i - \tilde{p} | / [2(n-1)/n]$. However, here it is important to notice, that this evenness measure is dependent from sampling size and representativity (Gregorius, 1990).

Table 1. An example of relative evenness and evenness deviation calculations for the allel frequencies in two natural populations of *Alnus acuminata* in Costa Rica

Allel	Bajos del Toro				Zarcero			
	IDH-A	MNR-A	PGM-A	PGI-B	IDH-A	MNR-A	PGM-A	PGI-B
1	0.836	1.0	0.108	0.025	0.772	1.0	0.100	0.036
2	0.164	0.0	0.067	0.934	0.228	0.0	0.056	0.821
3	—	—	0.825	0.041	—	—	0.844	0.143
Relative evenness (e)[1]	0.672	1.0	0.650	0.869	0.544	1.0	0.689	0.643
Evenness deviation	0.670	1.0	0.740	0.900	0.540	1.0	0.760	0.730

[1] Relative evenness $e = 1 - 2d_{min}$ (Gregorius, 1990).

This measure accounted for all the dispersion of observed allele frequencies from a hypothetical evenness frequency, which is an easier way to understand this concept. In the other hand, evenness from Gregorius (1990) and specially relative evenness ($e = 1 - 2d_{min}$, in Gillet, 1994; being d_{min} the minimal deviation) produces a value that theoretically should show that as e approaches a lower bound of 0.5, unevenness increases.

However, as can be seen from Table 1, the evenness values show exactly the opposite. The relative evenness produced a value of 0.672 for population Bajos del Toro at IDH-A which is much higher than the 0.544 value for population Zarcero in this same gene locus. That should indicate that Bajos del Toro approaches better to evenness, which is exactly the opposite to the observed allele frequencies. However, as can be seen from the same table, the relative evenness produces exactly the same values as those obtained with the evenness deviation parameter for gene loci with two alleles. For gene loci with more than 2 alleles there is a discrepancy between both parameters. In reality, the relative evenness parameter joints all alleles with the lower frequencies in one single allele, and then reproduces a new muster with 2 alleles. If the same approach is followed with the evenness deviation parameter, then there are produced exactly the same values. Clearly this procedure will produce lower values due to the average effect being promoted and therefore, some loss of information. Finally, since these evenness deviation values are relative and scaled from 0 to 1.0, an arithmetical mean (gene pool value) through gene loci can be produced.

The 17 populations were then graphically analyzed, contrasting the gene pool diversity and gene pool evenness deviations values, as well as diversity and potential genotypic diversity ($P_{Div} = N_1 * N_2 * \ldots * N_n$, being N_n = effective number of alleles in isozyme n). In each case diversity (x axis) against the other variable (y axis) were plotted and the culling levels ($\bar{x}$) for each variable as a reference guide for population selection were then utilized.

Results and discussion

Election of populations for gene conservation purposes

In tables 2 and 3 are showed the genetic parameters for both, the 17 populations and the 5 regions respectively. Among all single populations occupies Boquete the first place in rank with respect to almost all quantitative parameters. But in terms of qualitative variables, like number of effective alleles and number of multilocus genotypes, appear populations from Poás I and Poás II regions first. Other populations with a good ranking position in their diversity values were Coronado 1400 and San Gerardo, from Irazú and Talamanca regions respectively. These results are consistent with the previous analysis of the genetic structure of these populations (Murillo and Finkeldey, 2000). In terms of the regions, Boquete and Poás II showed consistently the higher values in almost all parameters. While Irazú region ranked invariably at lowest in all cases (Table 3).

Interesting is to look at qualitative parameters. Since the rare alleles do not cause major effect in the estimates of most of the quantitative parameters (Finkeldey and Gregorius, 1994; Hattemer, 1995), then it is important to add other parameters in the screening of populations for gene conservation. The number of effective alleles above a certain minimal frequency may be an important parameter in this direction, since they may also reflect an important adaptative effect

Table 2. Genetic parameters for 17 natural *Alnus acuminata* populations from Costa Rica and Panama (Murillo and Finkeldey, 2000)

Population	Diversity v	Multi-locus diversity v	Number of effective alleles		Potencial genotypic diversity		Number of multilocus genotypes		Sub-populat. different.	Eveness (e)[2]			Degree of heteroz.
			p ≥ 0.02	p ≥ 0.03	P_{Div} p ≥ 0.02	P_{Div} p ≥ 0.03	No.	n/No.[1]	D_j	IDH	PGI	PGM	
1. Zarcero	1.32	8.00	8	8	12	12	17	3.64	0.071	0.833	0.762	0.803	0.24
2. Bajos Toro	1.21	3.93	9	9	18	18	16	3.81	0.098	0.836	0.934	0.825	0.15
3. Vara Blanca	1.47	11.19	8	8	12	12	21	2.90	0.104	0.927	0.717	0.787	0.34
4. Cartagos	1.34	7.94	9	7	24	8	15	4.07	0.070	0.921	0.746	0.943	0.22
5. Coronado ≤ 1,400 mosl	1.38	7.45	8	8	16	16	11	3.91	0.080	0.988	0.823	0.872	0.24
6. Coronado > 2,000 mosl	1.25	5.14	7	7	8	8	12	5.00	0.094	0.898	0.850	0.992	0.19
7. LlanoGrande	1.29	6.50	7	7	8	8	9	6.67	0.081	0.975	0.792	0.958	0.22
8. Irazú	1.16	2.98	6	5	4	2	5	9.60	0.113	0.902	0.989	0.979	0.14
9. Pacayas	1.22	4.57	7	7	8	8	10	6.20	0.074	0.847	0.879	0.968	0.18
10. Turrialba	1.2	3.88	7	6	8	4	9	5.55	0.166	0.750	0.850	0.980	0.13
11. El Empalme	1.23	4.35	8	7	16	8	11	5.73	0.059	0.823	0.817	0.944	0.20
12. Cañón	1.27	5.43	8	8	16	16	13	4.69	0.045	0.779	0.754	0.949	0.23
13. Copey	1.29	5.52	7	7	8	8	13	4.85	0.112	0.929	0.841	0.817	0.22
14. SanGerardo	1.38	8.66	7	7	8	8	14	3.43	0.051	0.760	0.865	0.865	0.29
15. Siberia	1.28	4.90	8	7	16	8	9	3.44	0.083	0.907	0.839	0.875	0.26
16. División	1.19	3.62	8	7	16	8	13	4.69	0.081	0.926	0.817	0.910	0.15
17. Boquete	1.52	10.57	8	8	12	12	16	2.19	0.153	0.757	0.955	0.838	0.36
Range	1.16-1.52	2.98-11.19	6-9	5-9	4-24	2-18	5-21	2.2-9.6	0.04-0.17	0.75-0.99	0.72-0.99	0.79-0.99	0.13-0.36
Average	1.29	6.15	7.64	7.23	12.35	9.65	12.53	4.74	0.090	0.868	0.837	0.900	0.22

[1] n = sampling size and No. = number of multilocus genotypes. [2] Evenness (e) after Gregorius (1990).

(Finkeldey and Gregorius, 1994). The single populations from Poás I and Poás II regions contain the highest number of effective alleles. Only in these populations were found some of the rare alleles and both possess between 90 to 100% of the overall effective alleles found in this study. Thus, based on these criteria, some of these populations are clear candidates for gene conservation actions. However, the

Table 3. Genetic parameters for 5 natural *Alnus acuminata* regions of occurrence in Costa Rica and Panama

Population	Diversity v	Multi-locus diversity v	Number of effective alleles		Potencial genotypic diversity		Number of multilocus genotypes		Sub-populat. different.	Eveness (e)[2]			Observed hetero-cigosity
			p ≥ 0.02	p ≥ 0.03	P_{Div} p ≥ 0.02	P_{Div} p ≥ 0.03	No.	n/No.[1]	D_j	IDH	PGI	PGM	(H_0)
1. Poás I	1.273	5.634	9	8	18	12	24	3.73	0.091	0.835	0.843	0.818	0.190
2. Poás II	1.411	10.881	8	8	12	12	26	3.48	0.061	0.924	0.731	0.870	0.280
3. Irazú	1.262	5.516	7	7	8	8	16	5.71	0.120	0.970	0.863	0.961	0.182
4. Talamanca	1.277	5.632	7	7	8	8	24	4.40	0.085	0.849	0.766	0.896	0.220
5. Boquete	1.518	10.573	8	8	12	12	16	2.19	0.153	0.757	0.955	0.838	0.364
Range	1.26-1.52	5.51-10.88	7-9	7-8	8-18	8-12	16-26	2.2-5.7	0.06-0.15	0.75-0.97	0.73-0.95	0.82-0.96	0.19-0.36

[1] n = sampling size and No. = number of multilocus genotypes. [2] Evenness (e) after Gregorius (1990).

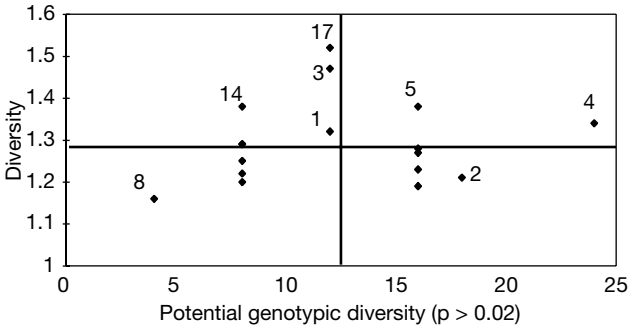

Figure 1. Election of *Alnus acuminata* populations for gene conservation purposes based on the relationship of their genetic diversity and potential genotypic diversity in Costa Rica and Panama.

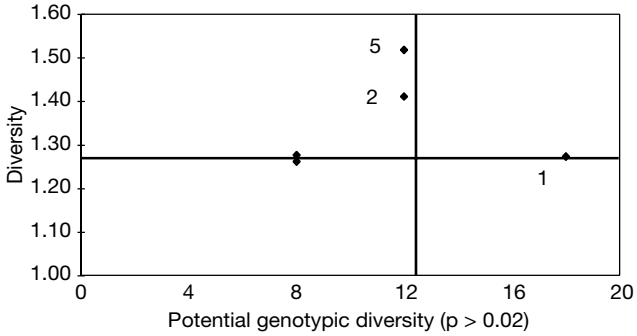

Figure 2. Election of *Alnus acuminata* regions for gene conservation purposes based on the relationship of their genetic diversity and potential genotypic diversity in Costa Rica and Panama.

presence of rare alleles is highly dependent on sampling size and sampling procedures. Thus, these type of parameters could cause easily a bias effect in the estimates obtained. A good example of this situation can be seen in population Los Cartagos (Table 2). Which showed a dramatic reduction (from 24 to 8) in the parameter potential genotypic diversity when being considered effective only those alleles with frequencies $p \geq 0.03$. This situation may partially explain the relative weak tendencies showed when plotted gene pool diversity against the potential genotypic diversity (Figures 1 and 2). In Figure 1 were found only populations 4 (Los Cartagos) and 5 (Coronado < 1,400 m) above both culling levels. Meanwhile, at the regional level were found none of the regions above both culling levels (Figure 2). Similarly, the parameter number of multilocus genotypes would have the same problems. But when the sample size is divided by the number of multilocus genotypes produces a better estimation of genetic richness as shown in the same Table 2. For instance, in Boquete population there is a probability of finding a new multilocus genotype each 2.19 trees, while in population Irazú there is a new multilocus variant almost after each 10 trees.

The evenness estimations were not very informative, since the values dispersion was not too large. Also, the values from most populations behave differently across loci. Should be also mentioned, that differences in observed allele number, within gene loci PGM-A and PGI-B, varied across populations and therefore, it may cause some differences in the number of plateaus considered by the evenness analysis (Hattemer *et al.*, 1993, page 281). In the other hand, the variable «evenness deviation» showed to be very informative (Table 4). The dispersion of values was large and consistent with the observed allelic frequency distributions. It provided also a gene pool value that could be plotted against diversity (Figures 3 and 4). In figure 3 can be clearly seen that populations 17 (Boquete), 3 (Vara Blanca), 5 (Coronado 1400), 14 (San Gerardo) and 4 (Los Cartagos) all have reached above both culling levels for diversity and evenness deviation, indicating jointly that these populations hold the highest genetic richness for gene conservation. In terms of regions, Boquete and Poás II showed the highest values and reached also both culling levels (Figure 4).

Another interesting parameter is subpopulation differentiation. In this case, can be as good a population which shares almost all their genetic elements with the rest of populations, than those populations which share the minimal (Hattemer, 1995). The latter case may indicate a high adaptation to local conditions and correspond typically to those populations located at the extremes of a natural distribution. In this study were found populations Boquete (at the extreme south), Vara Blanca (at the northern edge) and Turrialba (at the eastern edge of Irazú region) with the highest values in this parameter, and populations Cañón and San Gerardo (both from Talamanca region, in the center of the natural distribution) with the lowest values. The high value of subpopulation differentiation for population Turrialba may reflect a recent and strong local bottleneck effect like previously discussed (Murillo and Rocha, 1999; Finkeldey and Murillo, 1999).

The degree of heterozygosity was higher in populations Boquete, Vara Blanca and San Gerardo as expected due to their higher diversity values (Figure 5). Higher heterozygosity values are indicators of higher recombination rates and therefore of a better allelic interaction and maintenance in a population. For gene

Table 4. Estimation of evenness deviation in 17 natural populations at 4 polymorphic gene loci of *Alnus acuminata* populations from Costa Rica and Panama

Region	Population	MNR-A		PGM-A		IDH-A		PGI-B		Gene pool	
		Populat.	Region	Populat.	Region	Populat.	Region	Populat.	Region	Populat.	Region
Poás I	Zarcero	1.00	1.00	0.60	0.73	0.66	0.67	0.62	0.75	0.72	0.79
	Bajos del Toro	1.00		0.74		0.68		0.89		0.83	
Poás II	Vara Blanca	0.98	0.97	0.58	0.74	0.14	0.15	0.58	0.60	0.57	0.62
	Cartagos	0.96		0.88		0.16		0.62		0.65	
	Coronado ≤ 1,400 m	0.90	0.98	0.74	0.92	0.02	0.06	0.64	0.73	0.57	0.67
	Coronado > 2,000 m	0.94		0.98		0.20		0.70		0.70	
Irazú	Llano Grande	1.00		0.92		0.04		0.58		0.63	
	Irazú	1.00		0.96		0.20		0.98		0.78	
	Pacayas	1.00		0.94		0.30		0.76		0.75	
	Turrialba	1.00		0.96		0.50		0.70		0.79	
	El Empalme	0.96	0.97	0.88	0.84	0.64	0.70	0.64	0.47	0.78	0.74
	Cañón	0.94		0.90		0.56		0.50		0.72	
Talamanca	Copey	0.98		0.64		0.86		0.32		0.70	
	San Gerardo	0.98		0.72		0.48		0.26		0.61	
	Siberia	0.96		0.76		0.82		0.32		0.71	
	División	0.96		0.78		0.86		0.64		0.81	
Boquete	Boquete	1.00	1.00	0.44	0.44	0.52	0.52	0.08	0.08	0.51	0.51
Range		0.9-	1.0-	0.44-	0.44-	0.02-	0.06-	0.08-	0.08-	0.51-	0.51-
Range		1.0	0.97	0.98	0.92	0.86	0.70	0.98	0.76	0.83	0.79
Average		0.97	0.99	0.79	0.73	0.45	0.48	0.58	0.51	0.69	0.68

conservation purposes is clearly desirable to have populations with not only higher degree of heterozygosity, but also a good distribution of the different heterozygosity levels, like showed in figure 5 for some populations. Here showed population Boquete the highest frequency at the highest degrees of heterozygosity (k = 2 and k = 3), which indicates once again the suitable genetic structure of this population for gene conservation purposes.

In general can be said that there is at least one good candidate population in each of the major geographical regions (breeding region) suitable for conservation purposes: Vara Blanca (Poás II), San Gerardo (Talamanca) and Boquete (Panama). Even though population Coronado 1400 (Irazú region) shows an adequate genetic structure for this purposes, it can not be advocated to conservation due to its proximity to Coronado town and to its relative small population size. Besides, in this region exists already the Irazú National Park (approx. 2,300 has in size, in Vargas, 1994), which is located at no more than 15 km distance from this population and close to Coronado > 2,000 m

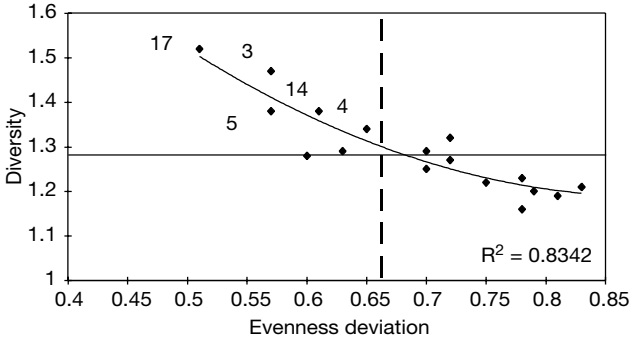

Figure 3. Election of *Alnus acuminata* populations for conservation purposes based on the relationship of their genetic diversity and evenness deviation in Costa Rica and Panama.

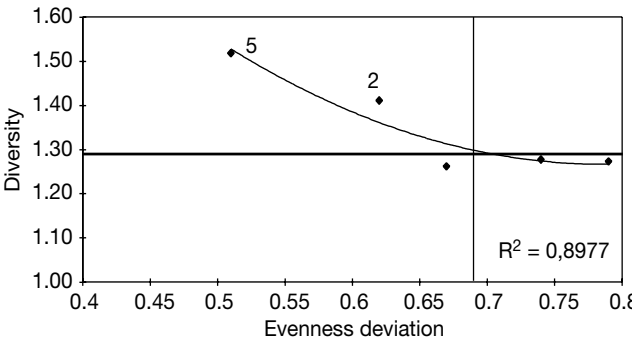

Figure 4. Election of *Alnus acuminata* regions for conservation purposes based on the relationship of their genetic diversity and evenness deviation in Costa Rica and Panama.

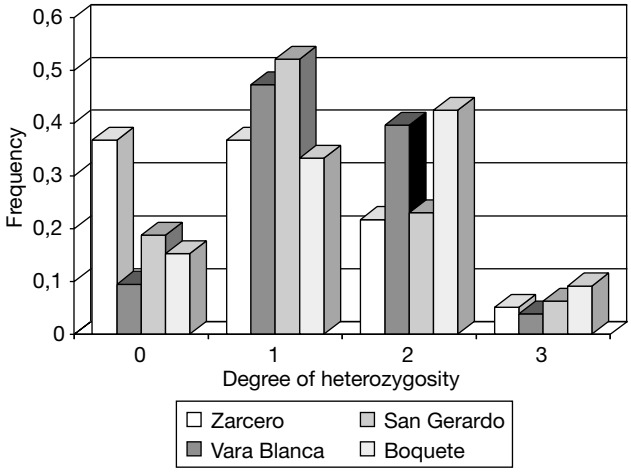

Figure 5. Distribution of the degree of heterozygosity in four selected populations of *Alnus acuminata* from Costa Rica and Panama.

population. In region Poás I the situation is optimal, since both populations are partially within the boundaries of the recently created Juan Castro Blanco National Park (approx. 14,000 has in size, in Vargas 1994). A similar situation occurs also within Talamanca and Boquete regions. In Talamanca region are situated the largest national parks from Costa Rica which comprise a big portion of the natural populations here investigated. San Gerardo population is surrounded by two large forest reserves (Los Santos Forest Reserve and Río Savegre private reserve) which ensure a good conservation conditions for this population. In the case of Boquete (Panama), the conservation status is unfortunately not secured. But this population is located at no more than 15 km from Barú volcano National Park and to the International Biosphere Reserve La Amistad, which runs along Costa Rica and Panama covering approx. 500,000 has (Vargas, 1994).

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