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SUBPOPULATION DIFFERENTIATION ALONG ELEVATIONAL TRANSECTS WITHIN TWO ITALIAN POPULATIONS OF SCOTS PINE (*PINUS SYLVESTRIS* L.)

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ABSTRACT

Two Italian populations of Scots pine (*Pinus sylvestris* L.) have been sampled along elevational transects at three different altitudes. The genetic structure of the six subpopulations has been studied by means of electrophoretic analysis of six enzyme systems (LAP, GOT, PGM, MDH, GDH, SKDH) for a total of 12 allozyme loci. An allele at locus *Got–B* shows the same type of clinal variation in both populations, consisting in a decrease of frequency at increasing altitude. Within each population, the differentiation between subpopulations appears clearly from the significant heterogeneity chi-square values at eight loci out of 12, suggesting a reduction of the gene flow as a consequence of asynchronous flowering due to thermic differences at different altitudes. Genetic diversity parameters show high values; the average values of expected heterozygosity are 0.299 and 0.295, respectively. The dendrogram based on Nei's genetic distances shows the ranking of a subpopulation from Lombardia in the cluster of Valle d'Aosta subpopulations, and some genetic distance values are much higher than that between the two populations as a whole. The obtained results clearly show the existence of differentiation within populations, and would allow to hypothesize an adaptive response of the allele *Got–B5* to the thermal gradient.

Key words: *Pinus sylvestris*, isozymes, subpopulation differentiation, elevational transects, clinal variation, gene flow.

INTRODUCTION

In the 70s, when protein electrophoresis was at its apogee, several attempts were made on various animal and plant organisms to investigate the physiological mechanisms explaining the conservation of enzymatic polymorphisms, based on the assumption that a high variability should have adaptive grounds. Such a selectionist approach was opposed by the neutralist theory, according to which evolutionary changes at the molecular level are caused mostly by the genetic drift effect on mutations – selectively neutral – generated at a constant rate as a consequence of nucleotide or amino acid substitutions. Therefore, most molecular polymorphisms would be the result of an equilibrium reached between the constant appearance of mutations and their random extinction. Only for few cases polymorphisms would have adaptive value and be submitted to the action of natural selection (HARTL & CLARK 1989; KIMURA 1989).

Indeed, starting from a selectionist approach, the risk is often of not evaluating objectively the available data and hypothesize the existence of cause-effect relationships between phenomena which are actually not connected among them. However, studies carried out on various animal and plant species demonstrate the existence of clinal variations in the allele frequencies at some enzyme loci and in some cases a direct relationship could be found between such clines and gradients of environmental variables, also identifying the physiological mechanisms which could explain the observed correlations. In numerous studies, correlations have also been observed between heterozygosity and growth rate (HEDRICK *et al.* 1976, LEDIG *et al.* 1983, HARTL *et al.* 1985, HARTL & CLARK 1989).

Many studies of this type have also been performed on forest tree species leading to contrasting results. These researches have been reviewed by MITTON & GRANT (1984), BUSH & SMOUSE (1992), SAVOLAINEN & HEDRICK (1995), and others. In particular, SAVOLAINEN & HEDRICK (1995) demonstrated that in Scots pine there is no association of fitness with heterozygosity.

Several studies have been carried out on the effects of pollution on forest tree population genetic structure (for a review on air pollution effects see SCHOLZ *et al.* 1989).

Among the species belonging to the *Pinaceae* family, Scots pine (*Pinus sylvestris* L.) covers the largest natural range and is one of the most important species for central-European and Scandinavian forestry.

For this reason it has been submitted to several investigations in different fields, including the study of population genetic structure through biochemical and, more recently, molecular markers. Hence, despite the present wide knowledge of Scots pine populations in large sectors of its range of distribution, information available on the European sector results to be incomplete since Italian populations have never been included in such investigations in the past.

To fill this gap, a research work has been carried out on eight natural populations representative of the Italian range of Scots pine, which covers Alps and northern Apennines (PUGLISI & ATTOLICO, submitted). Within two of these populations, three altitudinal belts have been sampled.

The present study aims to verifying the existence of possible differentiation between the sampled subpopulations in the two above-mentioned populations and to highlight the possible relationship between such a differentiation and altitude micro-environmental variations, mainly temperature gradient, in order to gain more detailed knowledge of the population genetic structure and assess the existence of a possible adaptive function for the studied enzyme systems.

MATERIALS AND METHODS

Sampled populations

Details on the two studied Alpine populations are given in Table 1. Both populations cover the southern slopes of the respective mountains.

Within each of three subpopulations per population, from 19 to 26 plants have been sampled at a minimum distance of 100–150 meters the one from the other, through cone collection. After extraction and treatment, the seeds of each mother plant, kept separate from those of the others, were stored in air-tight containers at a temperature of 3–4°C till analysis.

Isozyme analysis

Isozyme analyses were performed by means of horizontal starch gel electrophoresis on haploid megagametophytes and embryos of 12 seeds per mother tree, in order to distinguish between male and female gamete in each embryo for a following mating system analysis (PUGLISI *et al*, in preparation).

Six enzyme systems, coded by 12 loci, were utilized: LAP (leucine aminopeptidase, E.C. 3.4.11.1), GOT (glutamate oxaloacetate transaminase, E.C. 2.6.1.1), PGM (phosphoglucomutase, E.C. 2.7.5.1), MDH (malate dehydrogenase, E.C. 1.1.1.37), GDH (glutamate dehydrogenase, E.C. 1.4.1.2), SKDH (shikimate dehydrogenase, E.C. 1.1.1.25).

Endosperms and embryos were separately homogenized in a buffer 0.08 M tris – 1.00 M HCl, pH 7.2 (MÜLLER-STARCK, pers. comm.).

Electrophoresis was performed using the following buffer systems:

a) electrode buffer: 0.06 M NaOH – 0.30 M boric acid, pH 8.2; gel buffer: 0.08 M tris – 1.00 M HCl, pH 8.7 (POULIK 1957, modified), for LAP and GOT;

b) electrode buffer: 0.135 M tris – 0.047 M citric acid, pH 7.0; gel buffer: 0.034 M tris – 0.012 M citric acid, pH 7.0 (SHAW & PRASAD 1970, modified), for the remaining enzyme systems.

Starch gel concentration was 11% for the former buffer system and 12% for the latter. Staining was performed according to MÜLLER-STARCK (1998, and pers. comm.)

The genetic control of the utilized enzyme systems was previously determined by MÜLLER-STARCK (1982a, 1982b, and pers. comm.; PUGLISI *et al.*, in preparation).

For each enzyme system, loci were designated by capital letters following the enzyme acronym, marking the most anodal zone of activity by the first letter. Within each locus, alleles were designated by numbers, starting from the fastest one.

Table 1. Geographic origin of the investigated populations.

Locations	Number	Number of sampled trees	Latitude (N)	Longitude (E)	Altitude (m)	Region
Morgex	1	59	45°46'	07°00'	1000–1400	Valle d'Aosta
Morgex – A	1A	20			1000	
Morgex – B	1B	20			1200	
Morgex – C	1C	19			1400	
Morbegno	2	75	46°10'	09°36'	250–1200	Lombardia
Morbegno – A	2A	26			250–300	
Morbegno – B	2B	23			500–800	
Morbegno – C	2C	26			1000–1200	

Genetic parameters

Computations were performed with BIOSYS–1 software (SWOFFORD & SELANDER 1989) on embryo genotypes, in order to use a larger sample than the one constituted only by mother tree genotypes, including also alleles from pollen pool. The contingency table chi-square test (SNEDECOR & COCHRAN 1967) was used in order to estimate the heterogeneity between population distributions of allelic frequencies.

On the basis of the estimated allele frequencies, the following parameters of genetic diversity (variation within populations) were computed: average number of alleles per locus (N); percentage of polymorphic loci (P) computed on the basis of 5% criterion, i.e. the percentage of loci whose more common allele has a frequency lower than 95%; genetic diversity (v; GREGORIUS 1978; MÜLLER-STARCK & GREGORIUS 1986), also called effective number of alleles (CROW & KIMURA 1970), whose average value per population is computed as the harmonic mean of single locus values; expected heterozygosity according to Hardy-Weinberg (H_e; NEI 1978); observed heterozygosity (H_o). Wright's fixation index ($F = 1 - H_o/H_e$; WRIGHT 1922) was computed in order to compare observed heterozygosities with panmittic expectations.

Also the following parameters of genetic differentiation (variation between populations) were computed:

- genetic diversity analysis (NEI 1973, 1975), which show the distribution of genetic diversity: H_t (total diversity), H_s (diversity within populations), D_{st} (diversity among populations, given as the difference between the two former parameters) and G_{ST} (relative degree of genetic differentiation, given as the ratio D_{st}/H_t);
- subpopulation differentiation (d; GREGORIUS & ROBERDS 1986), which represents Gregorius' genetic distance between each population and the remaining ones, considered as a whole, and is regarded as more sensitive than G_{ST};
- genetic distance, computed by means of NEI's (1978) and GREGORIUS' (1974) formulae.

Values of Nei's genetic distance were used for constructing a dendrogram using the UPGMA method (SNEATH & SOKAL 1973).

RESULTS AND DISCUSSION

The frequency of some alleles show clinal variations, which occur in both populations only at locus *Got–B* (Table 2): allele 5 exhibits the same type of variation in both populations, consisting in a decrease of frequency at increasing altitude. Other alleles at this and at other loci (*Got–C, Pgm–A, Mdh*–C, *Mdh–D* and *Gdh–A*) also present a clinal frequency variation but only in one of the two populations. An adaptive origin could therefore be hypothesized for differences in subpopulation allele frequency for allele 5 at locus *Got–B*. Such a hypothesis could be extended to other cases of clinal variation only if other environmental factors are admitted to contribute, together with temperature, to triggering a different adaptive response in the two populations. The response could affect the observed loci directly, or any other loci tightly linked to them, as well as any coadapted gene complexes that they mark.

GOT is an enzyme catalyzing oxidative degradation of aspartic acid, an amino acid which when deprived of the amine group – transferred to the a-chetoglutaric acid which then transforms into glutamic acid – transforms into oxaloacetic acid. As regards a possible physiological mechanism explaining the dependence of allele frequencies of this enzyme on environmental thermic variables, we could hypothesize some kinetic differences among alleles, depending on different temperature values at which they express their maximum reaction velocity (BERGMANN 1978; MITTON & GRANT 1984), but this hypothesis would require some experimental bases before being taken into consideration.

GOT is included in group I enzymes, which are involved in the primary metabolism and normally use one particular substrate. These enzymes – especially the polymeric ones like GOT – tend to be less variable than non-specific group II enzymes, and are likely to be more sensitive to natural selection pressures. As a matter of fact, the optimally adapted three-dimensional structure of their molecules can be heavily altered by amino acid substitutions and, as a consequence, also their catalytic capacity can change, increasing or decreasing the organism fitness (GILLESPIE & LANGLEY 1974; BERGMANN 1991).

In the temperate zone mean annual temperatures decrease with a trend of about 0.6 °C per 100 meters of altitude (PIUSSI 1994). As a consequence, in the altitudinal range of the first population (Morgex, Valle d'Aosta) the mean temperature variation should be about 2.4 °C (1.2 °C between adjacent subpopulations), and in the second population (Morbegno, Lombardia) the variation should be about 5.7 °C (2.3–2.7 °C between adjacent subpopulations).

In each population, some subpopulations differ from the others for the presence or absence of rare alleles, which could be caused by sampling mistakes, despite this is a remote possibility on account of the large size of analyzed samples. These frequencies, too, could be originated by natural selection, mainly as far as rare alleles with deleterious effects are concerned, which could have disappeared where the selection

Table 2. Allele frequencies at the 12 analyzed enzyme loci.

Locus	Allele	Subpopulation					
		1A	1B	1C	2A	2B	2C
Lap–B	1	.033	.029	.037	.002	.044	.006
	2	.795	.892	.846	.992	.912	.968
	3	.172	.079	.117	.006	.044	.026
Got–A	1	.000	.010	.007	.000	.000	.000
	2	1.000	.990	.993	1.000	1.000	.997
	3	.000	.000	.000	.000	.000	.003
Got–B	1			.002	.053	.022	.006
	2	.063	.075	.147	.021	.009	.026
	3	.132	.154	.127	.174	.168	.248
	4	.109	.140	.197	.101	.176	.133
	5	.696	.631	.526	.643	.625	.587
	7	.000	.000	.000	.008	.000	.000
Got–C	1	.000	.000	.000	.002	.000	.002
	2	.263	.270	.238	.371	.243	.322
	3	.000	.008	.013	.031	.016	.013
	4	.658	.720	.749	.576	.726	.659
	5	.078	.002	.000	.021	.014	.005
Pgm–A	1	.058	.069	.048	.039	.053	.042
	2	.889	.837	.934	.925	.901	.870
	3	.053	.094	.018	.036	.046	.088
Mdh–A	1	.036	.100	.053	.056	.067	.067
	3	.964	.900	.945	.944	.933	.933
	4	.000	.000	.002	.000	.000	.000
Mdh–B	1	1.000	1.000	1.000	1.000	1.000	.998
	3	.000	.000	.000	.000	.000	.002
Mdh–C	1	.029	.006	.002	.002	.033	.010
	3	.707	.690	.780	.637	.701	.646
	4	.264	.304	.216	.361	.266	.345
	5	.000	.000	.002	.000	.000	.000
Mdh–D	1	.151	.154	.214	.223	.246	.202
	2	.274	.313	.396	.247	.310	.296
	3	.020	.004	.000	.005	.000	.018
	4	.555	.529	.390	.526	.443	.484
Gdh–A	1	.431	.406	.393	.429	.402	.425
	2	.569	.594	.607	.571	.598	.575
Skdh–A	2	.039	.019	.033	.040	.032	.048
	3	.777	.794	.783	.737	.805	.660
	4	.157	.175	.171	.201	.112	.263
	5	.026	.002	.011	.023	.051	.029
	6	.000	.010	.002	.000	.000	.000
Skdh–B	1	.000	.000	.000	.000	.006	.000
	2	.056	.108	.061	.041	.106	.056
	3	.944	.890	.937	.955	.885	.944
	4	.000	.003	.003	.003	.003	.000

pressure was stronger.

The hypothesis of an adaptive function of some enzyme loci or of their linked loci in forest trees has been formulated by several authors, among whom MITTON *et al.* (1977), BERGMANN (1978), LUNDKVIST (1979), YEH & LAYTON (1979), MITTON *et al.* (1980), YEH & O'MALLEY (1980), SCHUSTER *et al.* (1989), LÖCHELT & FRANKE (1995), KARA *et al.* (1997). In particular, BERGMANN (1978) managed to highlight a clinal variation of allele frequencies of acid phosphatase locus *Aph–B* along similar climatic gradients by studying Norway spruce populations (*Picea abies* Karst.) located along two transects, namely an elevational transect in the Austrian Alps and a latitudinal one in Finland, together with a group of Swiss populations not situated along any transect but living at different altitudes. In this investigation, the action (either direct or indirect) of natural selection – mainly exerted by temperature – on the alleles of an enzyme locus is clearly shown. The author puts forward the hypothesis of a possible physiological mechanism explaining the adaptive function of acid phosphatase.

There are, instead, papers showing that the used loci are mainly neutral to selection in conifers, as observed by ALDEN & LOOPSTRA (1987), MORAN *et al.* (1988), MORAN & ADAMS (1989), DIEBEL & FERET (1991) and others for various conifers.

The differentiation between subpopulations within each population appears clearly from heterogeneity chi-square values calculated on allele frequencies. Such values are significant in eight loci out of 12 in both populations (Table 3). On account of the short topographic distance between the subpopulations, these significant differences would suggest a reduction of the gene flow as a consequence of asynchronous flowering due to differences in temperature at different altitudes (MITTON *et al.* 1980, SCHUSTER *et al.* 1989), or to a limitation of the gene flow effects due to selection factors. In the former hypothesis, the gene flow would essentially take place through dissemination.

Genetic diversity parameters have high values (Table 4). In both populations, mean expected heterozygosity (H_e) is higher than the average value of expected heterozygosity within populations (0.151) estimated for gymnosperms by HAMRICK *et al.* (1992). The analyzed populations, and their subpopulations, are as variable as the Scots pine natural populations studied by other Authors in several European and Asiatic countries (MEJNARTOWICZ 1979; GULLBERG *et al.* 1982; MEJNARTOWICZ & BERGMANN 1985; KINLOCH *et al.* 1986; MUONA & HARJU 1989; SAVOLAINEN & YAZDANI 1991; WANG *et al.* 1991; GONCHARENKO *et al.* 1994; NEET-SARQUEDA 1994; PRUS-GŁOWACKI & STEPHAN 1994; SAVOLAINEN & HEDRICK 1995; SZMIDT *et al.* 1996). Heterozygosity values, both expected and observed, and v parameter (genetic diversity) show a clinal variation in the second population and tend to grow at increasing altitudes. On the contrary, in the first population observed heterozygosity decreases at increasing altitudes.

Table 3. Heterogeneity chi-square values computed on allelic frequency distributions of the studied subpopulations (D.F.: degrees of freedom; *: P<0.050; ***: P< 0.005). A): Values relative to population 1. B): Values relative to population 2.

A)

Locus	χ^2	D.F.	Significance level
Lap–B	19.575	4	***
Got–B	45.382	6	***
Got–C	43.710	4	***
Pgm–A	28.666	4	***
Mdh–A	17.506	2	***
Mdh–C	23.390	4	***
Mdh–D	29.821	4	***
Skdh–B	9.208	2	*
Total	217.258	30	***

B)

Locus	χ^2	D.F.	Significance level
Lap–B	56.744	4	***
Got–B	57.773	8	***
Got–C	36.879	6	***
Pgm–A	18.858	4	***
Mdh–C	32.672	4	***
Mdh–D	24.035	6	***
Skdh–A	50.405	6	***
Skdh–B	17.109	4	***
Total	294.475	42	***

Values of fixation index (Table 4) are positive in all the subpopulations, with high values in 1B and in the three subpopulations of population 2. They show a deficiency of heterozygotes relative to expected frequencies in panmittic equilibrium. As observed in many other conifers, which – contrary to angiosperms – lack any prezygotic incompatibility mechanisms (MÜLLER-STARCK & GREGORIUS 1988), Scots pine is a partially self-pollinating species (MÜLLER-STARCK 1977, 1979, 1982a, 1982b; YAZDANI *et al.* 1985; RUDIN *et al.* 1986; LONGAUER *et al.* 1992; KÄRKKÄINEN & SAVOLAINEN 1993; KÄRKKÄINEN *et al.* 1996). Plants originating from self-pollinated seeds are usually characterized by reduced viability (inbreeding depression), due to lethal

Table 4. Parameters of genetic diversity. N – mean number of alleles per locus; P – percentage of polymorphic loci at the 5% criterion; v – genetic diversity; H_e – expected heterozygosity according to Hardy-Weinberg; H_0 – observed heterozygosity; F – fixation index.

Subpopulation	N	P	v	H_e	H_0	F
1A	2.7	75.0	1.424	.298	.269	.097
1B	3.0	83.3	1.444	.309	.266	.139
1C	3.1	83.3	1.409	.291	.263	.096
Mean pop. 1	2.9	80.5	1.426	.299	.266	.111
2A	3.1	66.7	1.396	.284	.241	.151
2B	2.9	83.3	1.419	.296	.251	.152
2C	3.1	75.0	1.438	.305	.265	.131
Mean pop. 2	3.0	75.0	1.418	.295	.252	.145

and semi-lethal alleles, and are consequently subjected to natural selection (MÜLLER-STARCK 1982b; YAZDANI *et al.* 1985; LUNDKVIST *et al.* 1987; MUONA *et al.* 1987; KÄRKKÄINEN & SAVOLAINEN 1993; Zhelev *et al.* 1994; Savolainen & HEDRICK 1995; KÄRKKÄINEN *et al.* 1996). Our values refer to embryo genotypic frequencies, which normally include a certain amount of inbred progeny. A detailed comparison between embryonic and adult phases will be published in a separate paper (PUGLISI *et al.*, in preparation).

Table 5 reports the parameters of genetic diversity analysis. Mean values of G_{ST} are identical in both populations. The heterogeneity of G_{ST} values, which reveals a varying differentiation among the subpopulations depending on the different loci, could support the hypothesis that the observed differences could be due to natural selection. Indeed, differentiation should affect all loci to the same extent if it was completely neutral to the selection pressure of environmental factors. Furthermore, genetic drift would hardly have a significant role in large stands undergoing a constant gene flow together with others at the same altitude. Also differentiation between subpopulations expressed by the parameter δ (Table 6) – considered to be more sensitive than G_{ST} – is variable among loci.

Genetic distance values by Nei and Gregorius (Table 7) between subpopulations are mostly similar and sometimes much higher than those between the two populations as a whole (0.003 and 0.041, respectively; PUGLISI & ATTOLICO, submitted). The dendrogram based on Nei's genetic distances (Figure 1) shows the ranking of the mean altitude subpopulation from Lombardia (2B) in the cluster of Valle d'Aosta (1) subpopulations. Actually, one of the lowest genetic distances is between subpopulation 2B and the intermediate one from Valle d'Aosta (1B). This could be due to some environmental similarity between the ecological stands of these subpopulations. Furthermore, the subpopulation at the highest elevation in Valle d'Aosta (1C) and that from the lowest elevation in Lombardia (2A) show a genetic distance value twofold (Gregorius' distance) or fourfold (Nei's distance) that between the two populations as a whole. These data further confirm the existence of differentiation within populations.

CONCLUSIONS

The obtained results do not allow us to infer certain conclusions on the possible adaptive role of the enzyme systems under analysis, also because of the limited number of sampled elevational belts for each population and to the short distance between them. Nevertheless, some important data have been highlighted. The clinal variation of allele 5 at locus *Got*–B in both populations would allow to hypothesize an adaptive response to the temperature gradient; and the similarity between the Lombardia 2B subpopulation and the Valle d'Aosta subpopulations would lead to the hypothesis of an evolutionary convergence due to similar micro-environmental conditions.

Although such results are limited and incomplete, they provide a further contribution to knowledge in an important and controversial topic. They also emphasize the importance of this kind of researches to better understand the genetic structure of populations and their dynamics, and for the development of more accurate *in situ* conservation strategies, by taking into account also subpopulation specific genetic features, as in the case of subpopulation 2B. As a matter of fact, since a gene resource can be defined as a collection of biological material containing specific genetic information or a defined range of variants of such information (ZIEHE *et al.* 1989), a rather differentiated subpopulation could hypothetically be declarated to be a gene resource needing specific conservation measures.

Table 5. Genetic diversity analysis. H_t – total diversity; H_s – diversity within populations; D_{st} – diversity among populations (H_t–H_s); G_{st} – relative degree of genetic differentiation (D_s/H_t). A) Values relative to population 1. B) Values relative to population 2.

A)

Locus	H_t	H_s	D_{ST}	G_{ST}
Lap–B	.271	.268	.003	.011
Got–A	.011	.011	.000	.003
Got–B	.568	.561	.008	.014
Got–C	.431	.428	.003	.007
Pgm–A	.207	.205	.003	.013
Mdh–A	.119	.118	.001	.012
Mdh–B	.000	.000	.000	–
Mdh–C	.405	.402	.003	.007
Mdh–D	.621	.612	.009	.014
Gdh–A	.484	.483	.001	.001
Skdh–A	.355	.355	.000	.001
Skdh–B	.141	.140	.001	.008
Mean	0.301	0.299	0.003	0.008

B)

Locus	H_t	H_s	D_{ST}	G_{ST}
Lap–B	.083	.081	.002	.021
Got–A	.002	.002	.000	.002
Got–B	.559	.556	.003	.006
Got–C	.475	.468	.007	.014
Pgm–A	.187	.186	.001	.006
Mdh–A	.119	.119	.000	.000
Mdh–B	.001	.001	.000	.001
Mdh–C	.457	.455	.003	.006
Mdh–D	.634	.632	.002	.004
Gdh–A	.487	.486	.000	.001
Skdh–A	.422	.414	.008	.018
Skdh–B	.134	.133	.002	.013
Mean	0.297	0.294	0.002	0.008

Table 6. Genetic differentiation between popula-tions (δ values). D_{ji} – differentiation values of single populations. A) Values relative to population 1. B) Values relative to population 2.

A)

Locus	Subpopulation			δ
	1A	1B	1C	
	D_{j1}	D_{j2}	D_{j3}	
Lap–B	.075	.072	.008	.052
Got–A	.009	.007	.002	.006
Got–B	.116	.044	.153	.103
Got–C	.086	.037	.068	.064
Pgm–A	.005	.074	.071	.050
Mdh–A	.042	.056	.016	.038
Mdh–B	.000	.000	.000	.000
Mdh–C	.028	.064	.084	.059
Mdh–D	.112	.056	.164	.110
Gdh–A	.031	.006	.025	.021
Skdh–A	.033	.034	.009	.025
Skdh–B	.032	.051	.022	.035
Gene pool	0.047	0.042	0.052	0.047

B)

Locus	Subpopulation			δ
	2A	2B	2C	
	D_{j1}	D_{j2}	D_{j3}	
Lap–B	.050	.068	.016	.043
Got–A	.002	.002	.003	.002
Got–B	.089	.069	.088	.082
Got–C	.114	.110	.024	.081
Pgm–A	.041	.017	.047	.036
Mdh–A	.011	.005	.006	.007
Mdh–B	.001	.001	.002	.001
Mdh–C	.053	.087	.029	.055
Mdh–D	.061	.072	.034	.055
Gdh–A	.015	.025	.009	.016
Skdh–A	.017	.133	.116	.088
Skdh–B	.040	.065	.022	.041
Gene pool	0.041	0.054	0.033	0.042

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Table 7. Genetic distance calculated following Gregorius (above the diagonal) and Nei (below the diagonal).

Population	1A	1B	1C	2A	2B	2C
1A – Morgex A	–	0.48	.061	.060	.055	.062
1B – Morgex B	.003	–	.056	.064	.038	.053
1C – Morgex C	.007	.005	–	.081	.050	.075
2A – Morbegno A	.007	.005	.012	–	.060	.039
2B – Morbegno B	.005	.002	.004	.006	–	.056
2C – Morbegno C	.007	.004	.009	.002	.005	–

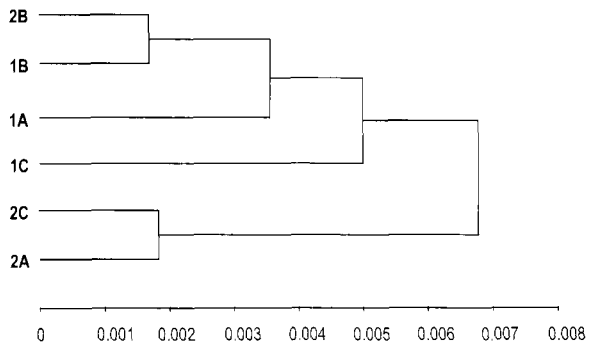

Figure 1. Dendrogram constructed on the basis of Nei's genetic distance values with UPGMA method.

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