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Population genetic structure of Alpine chamois (Rupicapra r. rupicapra) in the Italian Alps

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Abstract

Analysis of the genetic diversity of the Alpine chamois in Italy was conducted using a pool of 26 microsatellite loci. We hypothesized that geographical features limiting dispersal, local temporary extinction due to poor management, and the impact of severe diseases gave rise to measurable levels of population structure and differentiation within the study areas. Clear genetic differences have emerged among the sampled groups. Some were consistent with an isolation-by-distance model. However, other mechanisms intervened in parallel in areas that, in addition to being peripheral to the main alpine ridge, had suffered from recent bottlenecks due to poor management. In such areas, genetic drift and a low rate of gene flow are likely explanations of the current genetic structure.

Key words: Alpine chamois, Rupicapra rupicapra, population structure, microsatellite.

Introduction

Chamois (Rupicapra spp, Linneus, 1758) is a mountain-dwelling ungulate belonging to the subfamily Caprinae. Rupicapra originated in Asia during the Miocene epoch and spread to Europe during the early or middle Pleistocene, moving westward along the mountain chains of the Alpine system. There are two species of chamois in Europe: R. pyrenaica, which is distributed in the Pyrenees, Cantabrians, and Apennines; and R. rupicapra, which roams from the Carpathians to the Alps. Within the Alps, two subspecies have been described: R. r. rupicapra, which ranges over most of the chain, and the localized R. r. carthusiana, which lives in the Chartreuse massif in the French Alps (Masini and Lovari 1988).

An increasing number of investigations have been devoted to defining the phylogeographic structure of chamois populations based on allozyme and/or DNA polymorphisms (Miller and Hartl 1986; Pemberton et al. 1989; Perez et al. 2002; Schaschl et al. 2003).

Pemberton et al. (1989) demonstrated an existing gene pool divergence between the two subspecies (R. r. rupicapra and R. r. carthusiana) that was greater than divergences among local populations of Rupicapra r. rupicapra. Another investigation, carried out on chamois in the Eastern Alps, reported reduced gene flow among regional populations (Miller and Hartl 1986). In eight geographical populations of the two Rupicapra species, Perez et al. (2002) observed a deep divergence between R. rupicapra and R. pyrenaica. In addition, genetic distances between population pairs of the same species were highly correlated with the geographical distance between mountain chains. These findings suggest that the history of the genus during Pleistocene glacial-interglacial periods was dominated by phases of expansion and contraction, leading to alternate contact and isolation of contiguous populations. The warm climate of the Holocene had definitely caused the isolation of populations occupying the tops of the different mountain ranges. More recently, studies of maternally and biparentally inherited markers in chamois from the Eastern Alps showed a marked substructuring of the maternal gene pool into regional mitochondrial DNA (mtDNA) phylogroups, with restricted gene flow between neighboring populations. The spatial pattern of mtDNA variability was interpreted as a result of immigration of chamois from different Pleistocene refuges surrounding the Alps after glacier withdrawal. However, the distribution of allele frequencies at nuclear markers did not result in a corresponding pattern of geographical differentiation, possibly due to sex-specific dispersal with higher levels of philopatry in females and tendency toward dispersal in males (Schaschl et al. 2003).

Chamois are currently well distributed over all the Italian Alps, from the Ligurian Alps (LA) in the South-west to the Giulie Alps (GA) in the East. The most recent overall census counted approximately 137,000 animals, with an average density of 4.6 head/100 ha (Carnevali et al. 2008). Little information is available about the genetic structure of the chamois population in the Italian Alps.

Colonization of the current range might have resulted from only one major cohesive population, or from isolated source populations with more or less distinctly differentiated gene pools. Afterwards, the combination of limited gene flow and small population size may have caused genetic differences to accumulate rapidly in geographically separated populations.

Recently, the genetic diversity may have been decreased by bottlenecks, or by local temporary extinction due to poor management, habitat reduction, and pathogens.

The purpose of this work was to analyze the genetic diversity and the population structure of the Alpine chamois in Italy. We hypothesized that the distinctive orographic features of the Italian Alps limited dispersal of the chamois populations and gave rise to measurable levels of population structure and differentiation. These aspects were investigated using a considerable number of microsatellite loci to analyze an extended sample from six different geographic areas.

Materials and methods

Sample locations and collection

A total of 209 animals were analyzed, representing six different sampling locations in Italy, from the Southwest to the East Alps (Fig. 1). The term areas referred to these geographical locations as defined by the SOIUSA (Suddivisione Orografica Internazionale Unificata del Sistema Alpino) (Marazzi 2005).

Based on historical records all samples came from native populations, never subjected to documented human–mediated restocking.

Muscle samples were collected from 204 culled chamois of both sexes and varying ages during five consecutive hunting seasons. Whole blood samples were collected from five additional live-captured chamois in Carnic Alps (CR). All samples were stored frozen at –18 °C until analyses.

Molecular techniques

Genomic DNA was extracted from approximately 25 mg of tissue using the NucleoSpin Tissue extraction kit (Macherey-Nagel, Dueren, Germany). Blood samples of 200 μL were processed using the NucleoSpin Blood Quick Pure method (Macherey-Nagel, Dueren, Germany).

A total of 39 ungulate-derived microsatellite loci were chosen such as (i) to be well spaced across the genomes of cattle, sheep, and goats; (ii) to be informative in the original species; and (iii) to give good scoring performance on the genetic analyzer with multiplex polymerase chain reactions (PCR).

PCR was performed in a total volume of 10 μL with approximately 100 ng template DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.04 to 0.25 μM of each primer depending on the marker, and 0.4 units HotStarTaq polymerase (QIAGEN, Milano, Italia). The PCR profile consisted of an initial activation step at 95 °C for 15 min, followed by 30 cycles of 94°C denaturation for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min. Cycling was followed by a final extension step at 72°C for 7 min. Amplifications were carried out using a GeneAmp PCR System 2400 thermal cycler (Applied Biosystems, Foster City, California).

Prior to data collection, 12 individuals chosen randomly from the overall sample set were screened for each marker (via simplex PCR) in order to provide information on peak pattern and rough allele size range. Individual loci that failed to consistently amplify were discarded.

Finally, five multiplex PCRs were developed using fluorescently labelled primers. Each PCR reaction was mixed 12:1 with a mixture containing Hi-Di formamide and Size Standard 350-Rox (Applied Biosystems, Foster City, California). The denatured samples were run on an ABI PRISM 310 Genetic Analyzer. Data collection, extraction and analysis were performed with the GeneScan Analysis software, version 3.1.2. Allele calling (using the Genotyper software 3.7) was always combined with visual inspection of each sample.

Choice of loci

Some loci that were difficult to score using multiplex PCR were re-analyzed with simplex PCR. Because some statistical analyzes require equilibrium populations, each locus in every area was tested for deviations from the Hardy-Weinberg equilibrium (HWE).

F_{IS}-statistics per locus and sampled areas and the significance of their non-zero values were both performed on Fstat 2.9.3.2 software (alleles were randomized among individuals within populations, nominal level per multiple tests was 0.001) (Goudet 1995). An additional test implemented by Micro-Checker 2.2.3 software (95% confidence interval, 10,000x) was employed to investigate the possible influence of null alleles, the effects of stat bands or the presence of large allelic drop out (van Oosterhout et al. 2004).

The presence of genotypic disequilibrium between loci, across all populations and within individual populations, was checked using exact tests with Genepop software (1000 dememorization steps, 500 batches, and 4000 iteration per batch) (Raymond and Rousset 1995).

Genetic diversity

The actual number of alleles (A) and allelic richness (R) were calculated using Fstat. The number of private alleles (A_P) and heterozygosities (H) were obtained by Genetic Data Analysis 1.0 (GDA) software (Belkhir et al. 1998).

All pairs of populations were compared for homogeneity of genetic variation (number of alleles and heterozygosity) using Wilcoxon's matched-pairs signed-rank test (GraphPad InStat software, San Diego, CA, USA). The test evaluates whether the difference in loci information between two populations differs significantly from zero. A value W (sum of all signed ranks) is provided which is positive (negative) if the first population of a pair has more (less) diversity than the other.

Departures from Hardy-Weinberg expectations (HWE) were examined using the F_{IS}-statistics for all chosen loci and for each population, as performed on the Fstat software. The Bonferroni procedure for multiple comparisons was applied (adjusted nominal level of significance at 5%).

Genetic differentiation

Allele frequency differences across and between sampled areas were tested with F_{ST}-statistics across all loci using the analysis of molecular variance (AMOVA) as implemented by Arlequin 3.11 software (Schneider et al. 2000). The deviation from the null hypothesis was tested with 10000 permutations.

Genetic relationships between population pairs were estimated using Nei's standard unbiased distance, Ds, and the chord distance, Dc (Nei 1978; Cavalli Sforza and Edwards 1967). Both distances assume that differences between populations arose from genetic drift. Ds was formulated for an infinite allele model of mutation with the effective size of each population remaining constant, and it performed well with regard to fine-scale differentiation among populations. Dc assumes that there is no mutation, that all allele frequency changes were due to genetic drift alone, and that sizes did not remain constant and equal over time in all populations. The matrices of the genetic distances were constructed using Génétix software; significant deviations from the null values were tested with 10,000 permutations. Correlation between the two genetic distances was estimated with GraphPad InStat software.

A pattern of genetic variation that could derive from spatial limitations to gene flow was also hypothesized as an isolation-by-distance pattern (that is, a decrease in the genetic similarity among populations as the geographical distance between them increases). The relationships among genetic and spatial distances were examined with a Mantel test (10,000 randomizations) for matrix correlation between genetic distance and logarithmic transformation of geographical distance

between all population pairs, as implemented by the Isolation by Distance Web Service (IBDWS) (Jensen et al. 2005). The geographical distances were calculated between central positions of the sampled areas, taking into account only trails across mountains (not linear distances).

The relationship between sampled areas and individuals were represented using the factorial correspondence analysis (FCA) performed by Génétix software (Lewis and Zaykin 2001).

The Bayesian methodology of Structure 2.0 software allowed us to determine the level of structure in the dataset independently of the sampled areas (Pritchard et al. 2000). An admixture model was assumed with correlated allele frequencies, as this configuration was considered best in cases of subtle population structure (Falush et al. 2003). In this model, individuals may have mixed ancestry as a consequence of admixtures and hybrid zones. To identify the number K (a priori unknown) of different clusters of origin of the sampled individuals and to assign the individuals to these clusters, 20 independent runs for each K value ranging from 2 to 10 were conducted (length of burning period and Markov chain Monte Carlo of 10,000). Based on the rate of change in the log probability of data between successive K values, the real number of clusters was detected using the statistic ΔK proposed by Evanno et al. (2005). The modal value of this distribution was assumed to be the true or uppermost level of structure. A proportion of membership (Q) was estimated, for each individual, as a fraction of its genome drawn from each of the K-inferred clusters.

Results

Choice of loci

Thirteen marker loci of the 39 analyzed were discarded because they (i) failed to be amplified in all or most samples, (ii) showed no variation, (iii) could not be combined in any multiplex PCR, or (iv) revealed a systematic deviation from HWE due to null alleles or scoring errors (Table 1).

Forty individual samples that failed to consistently provide amplification products at all or most loci were dropped from the dataset.

The data on 26 marker loci of 169 individual samples from the six sampled areas were finally obtained (Table 2). The rate of missing genotypes was 0.8%.

Tests for genotypic disequilibrium revealed significant linkages when four sampled areas were pooled. No cases of identical linkage ware observed when the sampled areas were individually analyzed. This means that linkage disequilibrium depended mainly on the pooling of different populations.

Genetic diversity

At the 26 marker loci, 253 alleles were found (Table 2). The number of alleles per locus ranged from 18 (CSRD247) to two (ETH2).

The genetic variation within the four main sampled areas was quantified using the descriptive statistics of Table 3. No loci were monomorphic in individual areas. The average number of alleles was very similar to the average allelic richness; therefore, differences in sample size across areas did not affect the genetic analyses. Most private alleles were observed in the Dolomites (DO) and Cottian Alps (CA).

The combined F_{IS} values were not significantly different from zero. Consequently, no area showed consistent levels of HWE disequilibrium across all loci.

The differences between areas were estimated using the data summarized in Table 3 for allelic richness and for expected heterozygosity (Table 4). The highest diversity values were found in the CA. The population in the DO was more variable than the population found in the Lombard Prealps (LP). The least variable area was the LP.

Genetic differentiation

Variation among the four main areas, as estimated by the global F_{ST} index, was highly significant (global F_{ST} = 0.102; $P < 0.001$). All pairwise F_{ST} indices contributed with highly significant differences. All loci except for two (ETH2 and KP006) contributed significantly to the overall differences.

The matrices of the Ds and Dc pairwise genetic distances are presented in Table 5. All pairs of the four areas were significantly different ($P<0.001$). The two measures showed very similar patterns and were highly correlated ($r = 0.92$, $P<0.01$).

The two nearest areas, LA and CA, showed the least genetic distance between them. Meanwhile, two quite geographically distant areas, like CA and DO, did not show the greatest genetic distance, while a strong differentiation was found for the intermediate LP area. The genetic and geographical distances were not significantly correlated (Ds: $r = 0.326$; Dc: $r = 0.635$). However, a highly significant relationship was detected after removing the LP area (Ds: $r = 0.949$, $P<0.0001$; Dc: $r = 0.999$, $P<0.0001$).

The FCA plot of individual genotypes is presented in Fig. 2. The overall dataset from the six different sampled areas was used for this analysis.

The distribution based on the Axis 1 (32% of global genetic diversity) supported a clear distinction of individuals based on their rough geographical origin, the Southwest Alps, from the LA to GA (Graian Alps) areas versus the Southeast Alps, from the LP to CR areas. The three western areas

widely overlapped. However, the Axis 2 (27% of genetic diversity) clearly separated DO from the LP but not from the CR. The third factorial component (Axis 3, 18% of the total genetic variation, not shown) provided no additional information.

Under the hypothesis of two clusters using the Bayesian analysis (Fig. 3, a), 15 runs out of 20 split the dataset into samples from Southwest Alps and from the Southeast Alps. Most individuals were assigned to a cluster with $Q > 0.9$, as demonstrated by the high average proportions of membership.

At $K = 3$ (Fig. 3, b), on 16 runs out of 20, all individuals but one from the LP consistently segregated in a specific cluster with $Q > 0.9$ (average proportion 0.985). On average, high membership proportions were maintained.

At $K = 4$, all runs but one showed the individuals from LA to split from the Southwest samples, forming a specific cluster with robust membership (average proportion 0.963) (Fig. 3, c). Such a situation was also obtained on all the $K = 5$ runs (Fig. 3, d); in addition, the small sample of CR split from DO. However, because the membership of the CR sample was small (average $Q < 0.9$), this split should be verified on a greater number of samples. The other sampled areas all segregated in specific clusters with $Q > 0.9$ average proportions of membership. It is remarkable that at $K = 5$, all runs showed the same pattern.

The modal value of the statistic ΔK stated that the uppermost level of population structure was at $K = 5$, which was also the value that gave the maximum of log-likelihood of K with the minimum of variance across repetitions (Pritchard et al. 2000; Evanno et al. 2005).

At $K > 5$, the assignment confidence fell quickly because clusters with low average proportions of membership appeared and some clusters had no individuals assigned with $Q > 0.9$. Most individuals from the LP area continued to cluster together with high proportions of membership. However, confidence of assignment for individuals belonging to CA and GA fell rapidly and these areas never cluster separately with high membership values.

The cluster including most of the LP was very robust confirming that this area was the most differentiated but also the most homogenous within the present dataset, whereas CA-GA seemed to include many ‘admixed’ individuals. DO showed an intermediate picture similar to that of the LP.

Discussion

The selected molecular markers must be considered genetically independent and provide a good tool for diversity analyses in chamois. It is reasonable to assume that our microsatellite pool

represents a useful tool to monitor and – in context – manage chamois across its native geographic range.

The present study reveals a similar or greater level of variation at microsatellite marker loci in the Alpine chamois compared with other ungulates such as the Alpine ibex, Capra ibex (Maudet et al. 2002); the Sardinian mouflon, Ovis aries musimon (Kaeuffer et al. 2006); and Dall's sheep, Ovis dalli (Worley et al. 2004). In particular, our estimate of the number of alleles per locus, number of private alleles, and coefficients of heterozygosity shows higher diversity than has previously been reported for the Alpine chamois (Pérez et al. 2002; Crestanello et al. 2009). This discrepancy may be due to the fact that the molecular marker set we used has a higher information content. Additionally, the present investigation is based on a greater number of individuals, and on a more detailed sampling focused on the Italian Alps.

Our global F_{ST} is similar to the value reported for Rupicapra representatives collected in different European countries by Pérez et al. (2002) (0.102 vs. 0.118). Levels of genetic variation within areas are quite variable, which may reflect the different history of each group.

The genetic distance measures for Dc and Ds indicate that significant differences of allele distribution and frequency exist across sampled areas, probably due to genetic drift. The Ds distance between CA and DO samples is very similar to the corresponding distance in chamois from the Western and Eastern Alps (0.18 vs. 0.15), which was also estimated by Pérez et al. (2002).

The FCA plot, as well as the Bayesian method of assignment, reveals a robust difference among at least three main clusters: the Western cluster, including the areas of Liguria and Piedmont regions (LA-CA-G7A); the Central cluster of Lombard Prealps (LP); and the Eastern cluster, which spans the Trentino, Veneto, and Friuli regions (DO-CR). This genetic structure is strongly supported by stringent topographical features.

The typical habitat of chamois is represented by alpine pastures over 1,000 meters. The range of Alps is disrupted by deep river valleys that separate mountains peaks and represent strong migration barriers. Human activitiesfurther reduce natural corridors increasing fragmentation of suitable habitats.

In particular, the Adige river valley stretches between the LP and the Eastern cluster, whereas the valleys of the Adda and Ticino rivers stretch between the LP and the Western cluster (Fig. 1). These basins appear to be important geographical and anthropogenic barriers to dispersal. It is likely that human activities have stronger effects in the chamois than in other species because adaptation to isolated peak habitat produces strong divergence among groups (Maudet et al. 2002; Worley et al. 2004).

The Western cluster seems less homogeneous than the others. The CA and GA populations always share the same cluster at K = 2 to 5, whereas the LA population splits from the Western cluster at K = 4. This arrangement could reflect a fine-scale substructure of the Western Alps populations.

The genetic distances and the Bayesian assignment show the LA population to be a distinct genetic pool, whereas populations from the LA, CA, and GA widely overlap when FCA plotting is used. This apparent contradiction may be a consequence of differences in sensitivity to fine substructuring among methods. It is important to stress that the Bayesian approach is not affected by the constitution or boundaries of sampled areas when no information on location origin is incorporated into the analyses as a priori information to be combined with the genetic data.

In 1948, the Ligurian Alps appeared to be devoid of chamois. Subsequently, a process of recolonization occurred from the Southern Maritime Alps, located between the LA and the CA (not included in the present investigation), and in the late 1970's 50-60 individual chamois were present. Some differences in genetic constitution may therefore exist between the group that relocated to the LA area and the more Northern cluster, which includes animals from both the CA and the GA. Also, and not alternatively, the small number of migrants could have produced a founder effect. In fact, allelic richness and heterozygosity are significantly lower in the LA population than in the Northern cluster.

As regards the LP, a strong difference from the remaining areas was found in spite of its intermediate location. The Ds and Dc distances between the LP and any other location are greater than expected from the geographical distances alone. If the LP area is removed from the dataset, then a significant isolation-by-distance pattern is detectable for the LA, GA, and DO. Theoretically, an isolation-by-distance pattern is expected if individual clusters have narrow dispersal ranges in relation to the overall species range, and this may reflect local equilibria between gene flow and random drift (Worley et al., 2004).

One possible explanation of the remarkable genetic difference between the LP population and populations from the other sampled areas may be the recent history of the former group, which was close to extinction after the 2nd World War and was still numbering less than 200 individuals in the 1970's (Tosi and Perco 1981). Since the 1980's, westward dispersal of chamois from contiguous valleys (Alpi Orobie) and improved management rapidly contributed to recovery of the population to current 1650 heads (Citterio et al. 2003; AA.VV, 2008). The LP may have suffered from possible impacts of the founder effect (which produced a loss of genetic diversity and, subsequently, strong differentiation) combined with geographical isolation from the other two clusters due to the flanking river basins. Alternatively, it may be hypothesized that different genetic characteristics of the source metapopulation disrupted the expected isolation-by-distance pattern.

Regarding the Eastern cluster, our results suggest poor substructuring within and between the DO and CR, which only separate at K = 5. A high rate of dispersion in this part of the Alps may be due to the absence of major barriers East of the Adige valley.

Other putative causes of genetic differentiation among areas must be considered.

Genetic effects of harvest on wild animal populations have been discussed (Festa Bianchet 2003; Fenberg and Roy 2007; Milner et al. 2007; Coltman 2008), and three major types of potential harvest-driven genetic change have been pinpointed, namely alteration of population structure, loss of genetic variation, and selective changes (Allendorf et al. 2008). Traits more likely to be affected by human-induced selection in harvested population have been reviewed (Allendorf and Hard 2009). Among sport-harvested Caprinae, examples are known of long-term studied populations in which intensive trophy hunting has negatively modified the frequency of selected phenotypes ( ). In turn, harvest favors those phenotypes – i.e. males with smaller horns or body mass – to which are usually assigned a lower mating, and therefore genetic, value (Coltman et al. 2003; Garel et al. 2007). Little information is available on such potential effects in R. rupicapra, in which horn size and body mass are not such different between individuals as in Ovis or Capra spp. (xxxxxxx). This implies that selection from distance of a certain phenotype (i.e. a young vs. a full adult buck) is not realistic for most chamois hunters under "normal" field conditions (xxxxxx). Moreover, (i) current legislation dealing with the harvest of Northern chamois in Italy is quite conservative, as demonstrated by the continuous increase of population size since the 1980s ( ); (ii) local harvest plans must be elaborated according to national guidelines preventing major demographic disequilibria and then approved (or modified) by superior technical organisms ( ). Based on the above remarks and the evidence that harvesting in the LP area (started in xxxx) is planned and carried out according to similar criteria as in the other sampled areas, we tend to discard the hypothesis that the genetic peculiarities of this group derives from recent management deficiencies.

Finally, diseases are recognized increasingly as factors influencing host genetic diversity because they drive rapid declines in the abundance of exposed host populations ( ). Examples of short-term evolutionary dynamics are available for a series of host-pathogen systems ( ). Between xxxx and xxxx , the LP chamois has been affected by a pneumonia outbreak which reduced the pre-epidemic stock by an estimated average of %, with local maxima up to % (Citterio et al., 2003). The deviation of the LP chamois from the isolation-by-distance model may be regarded as a measurable effect of this recent bottleneck combined with the previously discussed population dynamics (geographical isolation and founder effect).

Nevertheless, we may notice here that a higher genetic diversity has been retained by the DO chamois despite having suffered from high mortality (49-77% decline) during a first epidemic wave

of scabies recorded since the mid-Nineties (Rossi et al. 2007). Apparently, the scabies-related population crashes have not resulted in a distinct substructuring of the investigated gene pool in the DO cluster. The other sampled areas Scabies has never been reported in Italy West of the Adige river (Berrilli et al. 2002; Schasch et al. 2003).

Conclusion

The present study documents extensive genetic variation in Italian chamois revealing the effects of natural and human habitat fragmentation and low migration rates on patterns of diversity.

Clear genetic differences have emerged between the sampled chamois groups. Expectedly, some were consistent with an isolation-by-distance model. However, in parallel, other mechanisms intervened in areas that, in addition to being peripheral to the main Alpine ridge, had suffered from recent bottlenecks. In such areas, genetic drift and a low rate of gene flow are likely explanations for the current genetic structure. Based on these results, it is reasonable to assume that our microsatellite pool and genetic dataset represent a useful tool to monitor and – in context – manage chamois across its native geographic range.

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Table 2 Genetic diversity found at the 26 marker loci used: number of alleles (A), allele size range, references and multiplex location.

Locus	A	size range (bp)	Reference	Multiplex
BM1258	16	82-140	Muntwyler et al., (2002)	2
BM1329	10	153-183	Muntwyler et al., (2002)	2
BMS332	11	126-152	Stone et al., (1995)	2
BMS4505	8	241-265	Muntwyler et al., (2002)	4
BOBT24	17	137-179	Muntwyler et al., (2002)	5
CSSM66	10	191-229	Muntwyler et al., (2002)	5
CSRD247	18	202-248	Muntwyler et al., (2002)	2
ETH2	2	195-197	Muntwyler et al., (2002)	4
ETH10	5	194-208	Muntwyler et al., (2002)	5
ETH225	7	128-150	Muntwyler et al., (2002)	2
FCB128	10	80-108	Muntwyler et al., (2002)	4
FCB20	9	75-101	Muntwyler et al., (2002)	5
FCB304	11	124-148	Muntwyler et al., (2002)	3
HSC	13	263-301	Muntwyler et al., (2002)	4
ILSTS005	10	156-188	Muntwyler et al., (2002)	1
ILSTS019	18	156-192	Muntwyler et al., (2002)	3
INRA005	9	115-137	Perez et al., (2000)	1
INRA011	14	200-230	Perez et al., (2000)	2
JMP029	6	120-142	Muntwyler et al., (2002)	3
KP006	8	192-206	Muntwyler et al., (2002)	4
NRAMP1	11	192-214	Muntwyler et al., (2002)	3
P019	4	161-167	Buitkamp et al., (1996)	4
SPS113	5	123-131	Muntwyler et al., (2002)	5
SRCRSP01	6	122-142	Perez et al., (2000)	5
SRCRSP05	7	154-172	Perez et al., (2000)	1
TGLA325	8	116-130	George et al., (1992)	2

Table 1 Discarded loci.

Locus	cause of discard
IDVGA46	no amplification
ILSTS029	no polymorphism
ILSTS030	null alleles
INRA040	null alleles
INRA063	no polymorphism
McM527	null alleles
SPS115	no amplification
SRCRSP06	no polimorphism
SRCRSP08	no coamplification
SRCRSP14	no amplification
SRCRSP15	no amplificatione
SRCRSP24	scoring error
TGLA263	null allele

Table 3 Genetic diversity estimates over 26 marker loci for each main sampled area included in this investigation.

	N	A	R	A_P	H_O	H_E	F_{IS}
LA	37	5.7	5.63	0.27	0.650	0.671	+0.031
CA	34	7.2	7.13	0.77	0.710	0.738	+0.038
LP	35	5.3	5.20	0.38	0.553	0.562	+0.018
DO	52	6.5	6.11	0.92	0.680	0.686	+0.008

Number of individual samples tested (N); average number of alleles per locus (A); average allelic richness (R); average number of private alleles per locus (A_P); average observed (H_O); expected (H_E) heterozygosity per locus; F_{IS} values over all loci.

Table 4 Wilcoxon's signed-rank test for allelic richness (above the diagonal) and heterozygosity (below the diagonal).

	LA	CA	LP	DO
LA	-	–277 ***	+114 n.s.	–114 n.s.
CA	–259 ***	-	+287 ***	+223 **
LP	+197 *	+295 ***	-	–170 *
DO	–83 n.s.	+175 *	–224 ***	-

n.s.: not significant - *: P<0.05 - **: P<0.01 - ***: P<0.001

The comparisons are always (first vs. latter) LA vs CA, LA vs LP, LA vs DO, CA vs. LP, CA vs DO, and LP vs. DO in both measures.

Table 5 Ds (above the diagonal) and Dc (below the diagonal) genetic distances between pairs of sampling areas.

	LA	CA	LP	DO
LA	-	0.138	0.350	0.228
CA	0.055	-	0.291	0.180
LP	0.126	0.107	-	0.273
DO	0.103	0.090	0.101	-

Fig. 1 Locations of samples used in this investigation: Ligurian Alps (LA 44° 05' 14" N; 7° 56' 33" E ⇒ 43° 57' 32" N; 7° 39' 07" E), Cottian Alps (CA 45° 06' 21" N; 6° 39' 41" E ⇒ 44° 52' 15" N; 6° 57' 09" E), Lombard Prealps (LP 46° 02' 32" N; 9° 21' 02" E ⇒ 45° 55' 25" N; 9° 28' 36" E), Dolomites (DO 46° 42' 37" N; 11° 52' 34" E ⇒ 46° 28' 33" N; 12° 27' 34" E) Graian Alps (GA 45° 23' 27" N; 7° 12' 28" E ⇒ 45° 30' 43" N; 7° 33' 29" E) and Carnic Alps (CR 46° 33' 55" N; 13° 21' 40" E ⇒ 46° 29' 07" N; 13° 41' 26" E).

Fig. 2 Distribution of individuals based on the FCA (boundaries of each sampled area are defined by lines surrounding all individuals kept within that area)

Fig. 3 Plot for the five sampled aereas. The graph is based on the STRUCTURE runs. Each individual is represented by a line partitioned into segments corresponding to its membership coefficients in the K inferred clusters, and each colour represents a different cluster. Black segments separate the individuals of different clusters. a: K=2; b: K=3; c: K=4; and d: K=5.