Delpinoa, n.s. **44**: 103-114. 2002

# Patterns of genetic variability in populations of *Adenostyles* Cass. complex (Asteraceae) along the Apennine chain

Anna Maria Palermo[1], Giuseppe Pellegrino[1], Maria Elena Noce[1], Liliana Bernardo[2], Aldo Musacchio[1]

[1]*Dipartimento di Ecologia,* [2]*Orto Botanico, Università della Calabria, I-87036 Arcavacata di Rende, CS, Italy.*

***Riassunto.*** *In popolazioni disgiunte di* Adenostyles *presenti lungo l'Appennino, catena montuosa che rappresenta un buon modello di gradiente eco-geografico, sono stati testati allozimi e RAPD. I RAPD sono risultati più variabili rispetto agli allozimi, giacché ogni individuo ha mostrato un genotipo differente dagli altri. Entrambi i marcatori hanno dimostrato che la diversità genetica maggiore è presente all'interno delle popolazioni, mentre i gruppi geografici sono scarsamente divergenti e non è stato riscontrato alcun gradiente geografico. La struttura genica spaziale non sarebbe da attribuire al flusso genico, ma piuttosto rappresenterebbe la conseguenza di contatti secondari. Questi eventi probabilmente avvennero durante i cambiamenti climatici del Quaternario, mentre la separazione fra le popolazioni esaminate sarebbe avvenuta di recente. In aggiunta, sono stati discussi gli aspetti conservazionistici, considerando sia la bassa consistenza delle popolazioni sia la riduzione dei loro ambienti.*

***Abstract.*** *Allozymes and RAPDs markers were tested in disjunct populations of* Adenostyles *complex along the Apennine chain, which is a good eco-geographic north-south gradient model. In our survey, RAPD was higher than allozyme variability, with each individual showing a different RAPD genotype. Both kinds of markers pointed out that majority of total genetic diversity resides within populations, while geographic groups are scarcely divergent and no clear geographic pattern was detected. Indeed, the dendrograms indicate that populations group together without congruence with their regional location. Data tend to exclude that this spatial genetic structure may be attributed to a constantly maintained gene flow, but rather could be explained as a consequence of secondary contact events. These events probably occurred during the climatic changes of Quaternary, and the separation of the examined populations seems very recent. A conservational point of view is discussed, considering the low number of individuals in these populations and the scarcity of their habitats.*

***Key words:*** Adenostyles*, allozymes, Apennine chain, Genetic variability, RAPDs, Spatial genetic structure*

## Introduction

In these last years, with the development of molecular markers, greater attention has been placed to phylogeographic studies on plants. In particularly, refugia and recolonization of arctic and alpine taxa have been investigated (i.e., Bauert *et al*., 1998; Stehlik *et al*., 2001), with the aim of substantiating or disproving the nunatakker theory (Stehlik, 2000). Independently from the occurrence of survivor populations on the Alps, few studies have been conducted on the behaviour of herbaceous species during glaciations in the Mediterranean basin (Comes & Kadereit, 1998), which has already proven to be an important refugium area for many tree species (Comes & Kadereit, 1998; Willis, 1996). In this context the Apennine chain, which has been viewed as an useful model of eco-geographic north-south gradient, represents a potential refugium for alpine species and, eventually, a

secondary colonisation route. In fact, its orophilous floristic component includes, among others, several Alpine species, capable of reaching different latitudes and that have spread along the chain presumably during the Quaternary climatic change. Due both to the peculiar orographic conformation and to the anthropic pressure, the populations of these species, living in disjunction with respect to their main areas of distribution, are often strongly isolated and progressively rarefied (PIGNATTI, 1980; PASSALACQUA & BERNARDO, 1997).

The study of spatial structure of these species with genetic markers may contribute to elucidate their evolutionary history and to shed light on the ecological and genetical influences of the colonisation and isolation events experienced by these taxa (THOMPSON, 1999; FREVILLE *et al.*, 2001). Moreover, recent occurrence of bottleneck or expansion in range may have significant effects on actual population genetic structure (HANNAN & ORICK, 2000). Unfortunately, there is a lack of interest towards the genetic structure of natural plant populations along the transet of this region (ZHANG *et al*., 2001; GRÜNANGER *et al*., 1998; FRIZZI & TAMMARO, 1991).

The genus *Adenostyles* Cass. is prevalently distributed throughout the Alpine territories, but its range extends also to Northern Spain, Corsica (France), Greece and along the entire Apennine chain in Italy (including mountains of Calabria and Sicily) (WAGENITZ, 1983; TUTIN, 1976). The Apenninic entities of *Adenostyles*, which occupy wet and shady habitats in beech forests above 1400 m a.s.l., represent a complex of strictly related taxa, similar to each other from both a morphological and ecological points of view. In the past, they have been regarded as varieties of an unique species, namely *A. alpina* (FIORI, 1925-29), whereas in more recent time they have been treated discordantly as different subspecies or species (PIGNATTI, 1982; WAGENITZ, 1983). In particular for these territories *A. glabra* (Mill.) DC., *A. australis* (Ten.) Nyman and *A. orientalis* Boiss. have been reported, although their differentiation has been based on few highly variable and quantitative characters. Infact, these species are notoriously difficult to determine and some reports of their presence are questioned (PIGNATTI, 1982). It has been shown that populations from central and northern zones of Apennines were erroneously referred to *A. glabra*, whereas they belong to *A. australis*. On the contrary, the alpine populations are referred to *A. glabra* and not to *A. australis.* (PIGNATTI, 1982). In any case, regardless of taxonomic implications Apenninic *Adenostyles* complex may represent a good model for exploring the pattern of genetic variation within and among populations of an Alpine element, progressively further from its main distribution area. As a matter of fact, its effectively linear scattering along a north-south environmental gradient may reveal patterns of gene flow or phylogeografic routes beside their taxonomic evaluation. Consequently, in this paper we chose to treat all examined populations as belonging to *Adenostyles* complex.

In addition, because no population genetic information exists on these species, genetic variability analysis may encourage future conservation measures. Knowledge of population genetic structure provides an historical perspective of evolutionary changes that characterise a species and allows to predict how populations will respond to future natural events (VRIJENHOEK, 1987; HUENNEKE, 1991; LUTZ *et al*., 2000). Consequently, the successful preservation of populations of rare,

endangered or fragmented taxa may greatly benefit from similar genetic variation results.

In this paper we investigate the allozymic and RAPD variability of populations of the Apenninic *Adenostyles* entities. These markers have been widely applied to describe the genetic structure and divergence existing within and among populations (HAMRICK & GODT, 1989; EDWARDS, 1998), with the aim of understanding the evolutionary factors responsible for their variation (SUN *et al*., 1999). RAPDs allow to emphasise variability in populations of endemic or rare plant species, even when allozymes failed in this goal (AYRES & RYAN, 1999).

In brief, the major objectives of the present study are a) to evaluate relative levels of genetic variation within Apenninic populations, b) to quantify differentiation among populations and gene flow, c) to assess genetic distances between populations in relation to their geographic distances, d) to indicate the potential consequences of disjunction and the causes responsible for the present *Adenostyles* distribution, e) to determine the possible conservational implications, since *Adenostyles* may be included at least among the rare species, considering the low consistency of their scattered populations and the relative scarcity of their habitats (CORBETTA *et al.*, 1998).

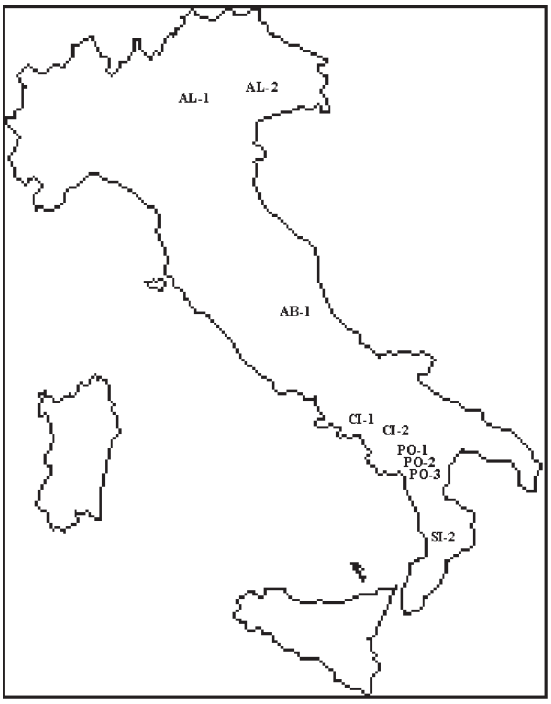

Fig. 1 - Geographic distribution of populations of *Adenostyles* complex along the Apennine chain

## MATERIALS AND METHODS

### *Plant material*

Samples were collected from two populations in alpine localities and from eight

Tab. 1 - Sampled populations of *Adenostyles*

| Sites | Region | Abbr. | N. of sampled analyzed | |
|---|---|---|---|---|
| | | | Allozyme | sRAPDs |
| Cozzo del Pellegrino | Pollino massif (northern Calabria) | PO-1 | 15 | 8 |
| Tavolara | Pollino massif (northern Calabria) | PO-2 | 15 | 8 |
| River | Pollino massif (northern Calabria) | PO-3 | 15 | 8 |
| Botte Donato | Sila (central Calabria) | SI-1 | 11 | 8 |
| Fontana Colonnello | Sila (central Calabria) | SI-2 | 6 | 6 |
| Cervati | Cilento (Campania) | CI-1 | 15 | 8 |
| Sicignano | Cilento (Campania) | CI-2 | 15 | 8 |
| Mount Velino | Marsica mountains (Abruzzo) | AB-1 | 11 | 8 |
| Magasa | Eastern Alps (Lombardia) | AL-1 | 15 | 8 |
| Trento | Eastern Alps (Trentino) | AL-2 | 4 | 4 |

populations along the Apennines chain (Fig. 1). All populations were constituted by a few specimens, but in all cases the numbers of studied samples were representative (Tab. 1). One or two leaves were collected from each of individuals per population and stored on ice in individually labelled plastic bags until they were transferred to a -80°C freezer, where they were kept until processed. We conducted RAPD assays on leaf tissue from a subset of the same individuals used for the isozyme analyses.

*Allozymes*

Enzymes were extracted by grinding leaf tissue with pH 7.5 Tris-HCl extraction buffer (SOLTIS *et al*., 1983), modified adding 1 mM PMSF. Crude extracts were absorbed on Whatmann 3MM paper wicks and stored at -80°C. Out of 20 enzyme systems tested, five were resolved by 10% horizontal starch gel electrophoresis, with the following buffer systems: Continuous Tris-Citrate (SHAW & PRASAD, 1970) for menadione reductase (MNR, EC 1.6.99.2) and Leucine aminopeptidase (LAP, EC 3.4.11.1), Tris-Versene-Borate (BREWER, 1970) for Acid phosphatase (ACP, EC 3.1.3.2), Phosphate-Citrate (HARRIS, 1966) for Malate dehydrogenase (MDH, EC 1.1.1.37) and Lithium Versene-Borate (SHAW & PRASAD, 1970) for Glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1). We selected these enzyme systems because they stained with sufficient intensity and resolution to be scored with confidence. Enzyme activity staining was carried out according to WENDEL & WEEDEN (1990) and BREWER & SING (1970).

Genetic interpretation of band patterns followed standard principles (WEEDEN & WENDEL, 1990; WENDEL & WEEDEN, 1990), taking in account that the chromosomic number of all *Adenostyles* is constant throughout the genus (2n=38) (FEDEROV, 1974).

*RAPDs*

Stored leaf tissue (approx. 0.3 g) of each sample was frozen in liquid nitrogen and ground into a fine powder. Total DNAs were then extracted according to DOYLE & DOYLE (1987).

RAPD markers were generated in 25 l containing 0.05 mmol of each of deoxynucleotide, 2 mmol MgCl2, 1x reaction buffer, 0.5 U Taq polymerase (Bioline USA, Inc), 0.04 mmol decamer primer and 10 ng sample DNA. PCR reactions were carried out, using primers supplied by Operon Technologies Inc. (Alameda, CA), in a 2400 Perkin-Elmer thermocycler, programmed for 40 cycles of 30 sec at 94 °C, 40 sec at 35 °C and 1 min at 72 °C. This was followed by 5 min at 72 °C. Amplification products were separated and visualised at 80 V for 2 h in 1,3% TBE agarose gels containing 0.5 g/ml ethidium bromide.

In a preliminary analysis 20 decamer primers were screened for suitability. Seven (OPD-05, OPD-06, OPD-07, OPD-08, OPD-11, OPD-12, OPD-18) were selected on the basis of best repeatability, clearness of the amplification pattern and their ability to reveal levels of diversity in *Adenostyles* specimens. Each DNA sample was amplified at least twice with all primers. Polymorphic bands were scored as present or absent.

## DATA ANALYSIS

*Allozymes*

The standard genetic parameters were calculated using TFPGA software (MILLER, 1997) to estimate allelic frequen-

cies, percent polymorphic loci at 95% criterion (P95), number of alleles per locus (A), expected and observed heterozygosity (He and Ho respectively) and departure from Hardy-Weinberg equilibrium for each populations.

Population substructure was estimated with Wright's F statistics (WRIGHT, 1965), FIT, FIS and FST, according to WEIR & COCKERHAM (1984), using the computer software FSTAT (GOUDET, 2000). These analyses indicate reductions in heterozygosity expected under random mating in individuals relative to subpopulations (FIS), or the total variation in the species (FIT), and the amount of variation distributed among populations (FST). Use of this method allowed us to test whether these F values were significantly different from zero.

Nei's (NEI, 1987) genetic identities, useful to evaluate the interpopulation differentiation, were obtained with the same program. A phenogram based on Nei's genetic distances was constructed using the unweighted pair group method with arithmetic averages (UPGMA) of SNEATH & SOKAL (1973) using TFPGA software.

*RAPDs*

Due to the dominant nature of RAPD markers, allele frequencies must be determined under the assumptions that only two alleles exist at a locus and that the populations are in Hardy-Weinberg equilibrium unless estimates of inbreeding are available from other data. Estimates of population differentiation GST were calculated under the assumptions of population equilibrium using POPGENE 32 software (YEH *et al*., 1997). For two alleles at a locus, as applicable in RAPD analysis, GST is identical to Wright's FST.

An analysis of molecular variance (AMOVA), using squared Euclidean distances, was carried out with AMOVA 1.55 software to partition the total variance into covariance components, and to find their significance levels, according to inter-individual and inter-population differences (EXCOFFIER *et al*., 1992) at the level of geographic region.

Shannon Information Index (I) to estimate variability at population and species level and Nei's (NEI, 1987) genetic identities were calculated using POPGENE 32 software (YEH *et al*., 1997).

Nei's genetic distances, calculated with the same software for all pairwise population comparisons, were used to construct the unweighted pair group method with arithmetic averages (UPGMA).

In addition, significant differences and correlation between the two molecular data set markers were evaluated using the two-tailed t-test and the chi-square test and the relationship between genetic distance and geographic distance was explored using the Mantel test (SOKAL & ROHLF, 1995).

## RESULTS

*Allozymes*

From the evaluation of 12 enzymatic systems, 5 have furnished reliable and repeatable bands. Five putative loci have been identified, each with 2 alleles. Monomorphic loci have not been detected, although a fixed allele of MNR enzyme has been observed in the Sila and Alps populations.

The number of alleles per locus, the percentage of polymorphic loci are reported in Tab. 2. Only three populations (PO-3, AB-1 and AL-1) have heterozytosity values that meet expected values under Hardy-Weinberg equilibrium, while two populations (SI-1 and SI-2) show negative

Tab. 2 - Summary of allozyme variation within populations of *Adenostyles*: percentage of polymorphic loci (P95), mean number of alleles per locus (A), observed heterozygosity (Ho), expected heterozygosity (He), standard error (SE).

| Site | P95 | A | Ho (SE) | He (SE) |
|---|---|---|---|---|
| Pollino massif (northern Calabria) | | | | |
| PO-1 | 80 | 1.8 | 0.250 (0.287) | 0.329 (0.246) |
| PO-2 | 100 | 2.0 | 0.274 (0.180) | 0.430 (0.105) |
| PO-3 | 60 | 1.6 | 0.257 (0.329) | 0.276 (0.256) |
| Population mean | 80 | 1.8 | 0.259 (0.265) | 0.345 (0.202) |
| Sila (central Calabria) | | | | |
| SI-1 | 80 | 1.8 | 0.415 (0.325) | 0.384 (0.219) |
| SI-2 | 20 | 1.2 | 0.120 (0.268) | 0.093 (0.209) |
| Population mean | 50 | 1.5 | 0.267 (0.296) | 0.238 (0.214) |
| Central Apennines | | | | |
| CI-1 | 80 | 1.8 | 0.121 (0.146) | 0.279 (0.213) |
| CI-2 | 80 | 1.8 | 0.254 (0.293) | 0.393 (0.222) |
| AB-1 | 100 | 2.0 | 0.431 (0.126) | 0.458 (0.037) |
| Population mean | 87 | 1.9 | 0.269 (0.188) | 0.376 (0.157) |
| Eastern Alps | | | | |
| AL-1 | 80 | 1.8 | 0.355 (0.342) | 0.397 (0.225) |
| AL-2 | 60 | 1.6 | 0.200 (0.274) | 0.257 (0.235) |
| Population mean | 70 | 1.7 | 0.277 (0.308) | 0.327 (0.230) |

fixation indexes, indicative of an excess of heterozygotes.

Wright F statistics are variable among the different loci, with all values always significantly different from zero ($p<0.001$). The partitioning of variability

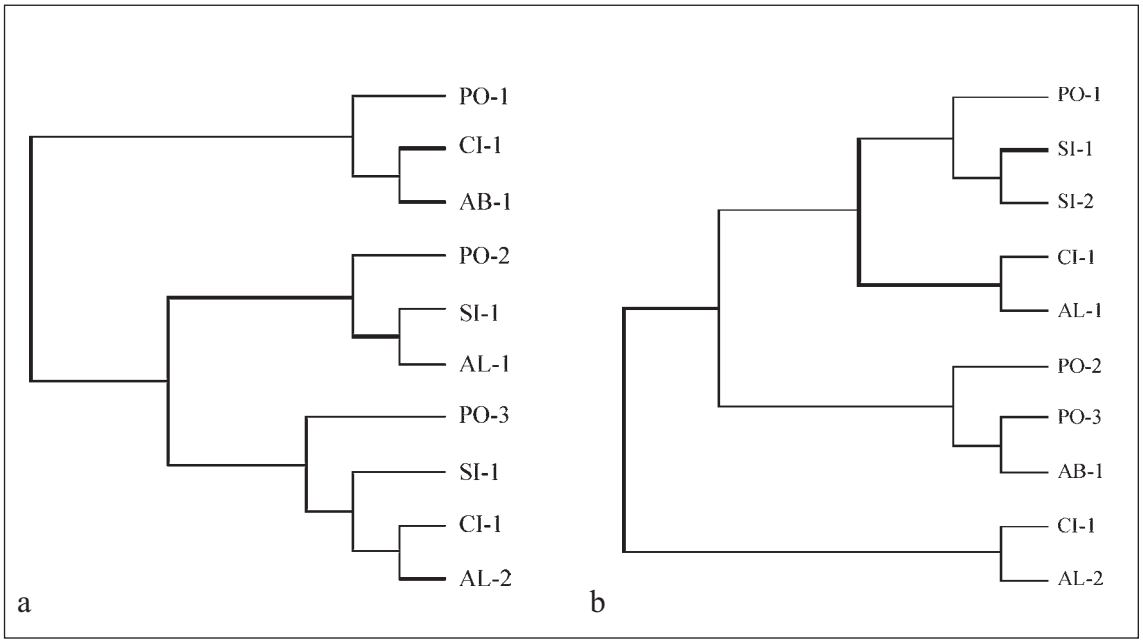


Fig. 2 - Allozyme (a) and RAPD (b) UPGMA dendrogram of Nei's genetic distance between populations of *Adenostyles*

within and among populations was also shown to be significantly different from zero ($p<0.001$). The mean Fst for polymorphic loci is 0.082.

The Nei's (NEI, 1972; 1987) genetic distances (ranging from 0.04 to 0.38) have been used to elaborate the similarity dendrogram (Fig. 2a), which does not indicate geographic structuring among the populations.

*RAPDs*

The seven primers used in the study allowed the scoring of a total of 45 loci, with an average of 6.4 loci per primer. Number of bands per primer ranges from 5 (OPD-6, OPD-18) to 11 (OPD-12) and their length from 370 to 1050 bp. We found a high amount of RAPD variation in the examined populations of *Adenostyles*, revealed by the unique RAPD multiband phenotype.

Nested AMOVA evaluations at geographic levels revealed a significant ($P<0.001$) genetic diversity within (approx. 67%) and among populations (approx. 32%), while resulting non significant between geographic regions ($P=0.7353$) (Tab. 3). From the AMOVA F statistics, analogue to the F statistics, it appears that interpopulation differentiation (Fst) at geographic level is higher (0.338) than that of narrowly distributed species with a mixed or outcrossing breeding system (0.2-0.28). All the pairwise values between populations are significantly different from zero, aside from AB-1 vs CI-1 ($P<0.05$) and PO-1 vs AB-1, which are negative.

Tab. 3- Summary of analysis of molecular variance (AMOVA)

| | | Variance component | | |
|---|---|---|---|---|
| Level of variation | Df | Absolute | % | P |
| Among geographic regions | 4 | -0.283 | -3.62 | 0.7353 |
| Among populations within geographic regions | 5 | 2.864 | 36.60 | <0.001 |
| Within populations | 66 | 5.243 | 67.02 | <0.001 |

Nei's genetic diversity values of population and geographic levels are indicative of a good variability, in accord to those obtained with various Nei's formulations in multilocus estimates, which may be biased by the dominant character of the RAPDs marker (NYBOM & BARTISH, 2000).

The RAPD dendrogram (Fig. 2b) does not resolve populations and geographic groups, indicating that intrapopulation polymorphisms exceeds interpopulation ones. Corroborating this, the Mantel's test indicated no correlation between genetic distance and geographic distance among samples. Otherwise, the Shannon Index (0.55), which is less influenced by the dominance of the markers, is indicative of similar variability patterns.

The $G_{st}$ value (0.40) is higher than that relative to perennial herbaceous plant species with a narrow (0.22) and regional (0.35) distribution (NYBOM & BARTISH, 2000). However, the correlation between the RAPD-based $G_{st}$ and the allozyme-derived $F_{st}$, is not significant (paired T-test = - 1.104; $0.1<p<0.375$).

## DISCUSSION

Evidences from both markers (RAPD and allozyme) indicate that *Adenostyles* populations distributed along the Apennine chain show a remarkable genetic diversity level, with the majority of the total diversity within the populations. No

evident pattern of spatial genetic structure has been detected and the geographic groups are scarcely divergent (HAMRICK & GODT, 1989; NYBOM & BARTISH, 2000).

The Apennine populations of *Adenostyles* used in the present study show a higher allozyme diversity than that usually found in rare species or in isolated populations at the border of the distribution area (ESSELMAN *et al*., 2000; GITZENDANNER & SOLTIS, 2000). At the same time, allozymic genotypes of the examined populations of *Adenostyles* appear to be rather homogeneous. They differ only for the relative frequencies of common alleles and, excluding the unique allele of MNR fixed in the individuals collected from Sila mountains and eastern Alps, suggest that all populations are part of a larger genetic pool.

The same populations exhibit greater variation at RAPD loci than at allozyme loci. RAPD variation is most apparent in different band frequencies among populations, and every individuals exhibited a unique multilocus phenotype. Usually, PCR-based DNA markers reveal equal or higher levels of genetic variability (WOLFE & MORGAN-RICHARDS, 1998; ESSELMAN *et al*., 2000). Greater diversity in DNA markers such as RAPD is attributed to the fact that many loci are amplified, providing a genome-wide survey (WILLIAMS *et al*., 1990). In contrast to allozyme loci, noncoding region, such as those that may be amplified with random primers, are unlikely to be under heavy selective constraints. Consequently, allozymes are considered more sensitive to selective pressures, while the RAPD variability depends primarily on evolutionary mechanisms, like mutations and drift (SUN *et al*., 1999).

However, in this case both allozyme and RAPD data have failed to find clear geographic patterns of the genetic variability in *Adenostyles*. Indeed, the relative dendrograms point out that populations group without congruence with their regional location (Fig 2a, b). A lack of congruence between genetic differentiaton among populations, which is low, and geographic pattern of population groups, has been reported for *Saxifraga oppositifolia*, an alpine taxon living in habitats with a small-scale heterogeneity. This finding has been attributed to a rare gene flow among relatively distant examined populations (GUGERLI *et al*., 1999).

In this respect, the high genetic diversity and variation found among the *Adenostyles* populations, does not suggest that gene flow is still occurring, but rather points out a common genetic exchange only (if at all) recently interrupted (SCHAAL *et al*., 1998). The discrepancy in levels and structuring of genetic diversity, as measured by allozymes and RAPD, undoubtedly, is the result of recent and historical genetic and ecological factors. Moreover, the lack of molecular gradients, being not correlated to geographic distribution, tends to exclude a history of migration and recolonization of the Apennine chain. Additionally, other episodes, like long distance dispersal or founder events, that usually imply a reduction of the genetic variability may account for this phenomenon (COMES & KADEREIT, 1998; THOMPSON, 1999). The UPGMA phenogram does not show a pattern of sequential relationships among populations from north to south, so excluding a scenario of unidirectional range expansion, but rather display a distribution of *Adenostyles* occurring via recent habitat fragmentation of a single and wide-spread ancestral entity distributed along whole Apennine chain. A plausible explanation could be that during the Quaternary *Adenostyles*, being part of

the vegetation that grew at the boundaries of glacial zones along Apennine chain, finally managed to establish contact with the wet habitats where they are at presently largely located (WILLIS, 1996). In this manner, these taxa underwent periodic expansion and retreats of their vegetational areas with connected episodes of secondary contacts, which could explain their present genetic similarity and the absence of any geographic gradient within Apennine populations (Fig. 1) (THOMPSON, 1999). As a consequence, in the postglacial period these populations became progressively isolated with the appearance of dryer conditions. In these isolated populations, the alteration of their pollinator set (CONTI *et al*., 2000) and/or the insurgence of autogamy (CRAWLEY, 1977) may have caused the little morphological diversification (i.e number of florets, WAGENITZ, 1983).

Such a scenario appears to be compatible with the aforementioned difficulty in discriminating taxa of *Adenostyles*, independently from each of the particular taxonomic proposal. It is more probable that these taxa have experienced some situations of parapatry and/or hybridization leading to genetic as well as to morphological homogenization.

Finally, one of the purposes of this work was to exploit molecular data in a biological conservation framework, an appropriate objective if one considers the low consistency and the disjunction of the examined populations (BARRET & KHON, 1991). Units appropriate for conservation and management should be established on the basis of their evolutionary potential. In this regard, variation at allozyme and RAPD loci in Apennine populations of *Adenostyles* complex may be interpreted as an indication of genetic potential, showing that Apennines populations of *Adenostyles* complex still have the possibility to persist and sustain future evolutionary change.

***Acknowledgements*** This paper was supported from a MURST PRIN (2000) grant.

## LITERATURE CITED

AYRES D. R. & RYAN F. J. 1999. Genetic diversity and structure of the narrow endemic *Wyethia reticulata* and its congener *W. bolanderi* (Asteraceae) using RAPD and allozyme techniques. American Journal of Botany, 86 (3): 344-353.

BARRET S. C. & KHON J. R. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. In: Falk D.A., Holsinger K.E. (Eds.). Genetics and conservation of rare plants. Pag. 3-30. Oxford University Press, New York.

BAUERT M. R., KAELIN M., BALTISBERGER M. & EDWARDS P. J. 1998. No genetic variation detected within isolated relict populations of *Saxifraga cernua* in the Alps using RAPD markers. Molecular Ecology, 7: 1519-1527.

BREWER G. J. & SING C. F. 1970. An introduction to isozyme techniques. Academic Press, New York & London.

COMES H. P. & KADEREIT J. W. 1998. The effects of Quaternary climatic changes on plant distribution and evolution. Trends in Plant Science, 3 (11): 432-438.

CONTI E., SURING E., BOYD D., JORGENSEN J., GRANT J. & KELSO S. 2000. Phylogenetic relationships and character evolution in *Primula* L.: the usefulness of

ITS sequence data. Plant Biosystems, 134 (3): 385-392.

Corbetta F., Abbate G., Frattaroli A. R. & Pirone G. F. 1998. S.O.S. VERDE! Vegetazioni e specie da conservare. Edagricole, Bologna.

Crawley M. J. 1977. Sex - Plant ecology. Pag. 156-213. Blackwell Science, Oxford.

Doyle J. J. & Doyle J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bullettin, 19 (1): 11-15.

Edwards K. J. 1998. Randomly amplified polymorphic DNAs (RAPDs). In: Karp A., Isaac P.G., Ingram D.S., Molecular tools for screening biodiversity. (Eds.). Plants and animals. Chapman & Hall London.

Esselmann E. J., Crawford D.J., Brauner S., Stuessy T. F., Anderson G. J. & Silva O. M. 2000. RAPD markers diversity within divergence among species of *Dendroseris* (Asteraceae: Lactucaceaea). American Journal of Botany, 87 (4): 591-596.

Excoffier L., Smouse P. E. & Quattro J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131: 479-491.

Federov F. 1974. Chromosome numbers of flowering plants. Otto Koeltz Science Publishers.

Fiori A. 1925-29. Flora Analitica d'Italia. Vol 2: 582-583. Edagricole, Bologna.

Freville H., Justy F. & Olivieri I. 2001. Comparative allozyme and microsatellite population structure in a narrow endemic plant species, *Centaurea corymbosa* Pourret (Asteraceae). Molecular Ecology, 10: 879-889.

Frizzi G. & Tammaro F. 1991. Electrophoretic study and genetic affinity in the *Campanula elatines* and *C. fragilis* (Campanulaceae) rock-plants group from Italy and W. Jugoslavia. Plant Systematic and Evolution, 174: 67-73.

Gitzendanner M. A. & Soltis P. S. 2000. Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany, 87 (6): 783-792.

Goudet J. 2000. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.1).

Grünanger P., Caporali E., Marziani G., Menguzzato E. & Servettaz O. 1998. Molecular (RAPD) analysis on Italian taxa of the *Ophrys bertolonii* aggregate (Orchidaceae). Plant Systematic and Evolution, 212: 177-184.

Gugerli F., Eichenberger K. & Schneller J. J. 1999. Promiscuity in populations of the cushion plant *Saxifraga oppositifolia* in the Swiss Alps as inferred from random amplified polymorphic DNA (RAPD). Molecular Ecology, 8: 453-461.

Hamrick J. L. & Godt M. J. W. 1989. Allozyme diversity in plant species. In: Brown A. H. D., Clegg M. T., Kahler A. L., Weir B. S. (Eds.). Plant population genetics, breeding and genetic resources. Pag. 43-63. Sinauer, Sunderland, Massachusetts, USA.

Hannan G. L. & Orick M. W. 2000. Isozyme diversity in *Iris cristata* and the threatened glacial endemic *I. lacustris* (Iridaceae). American Journal of Botany, 87 (3): 293-301.

Harris H. 1966. Enzyme polymorphism in man. Proceedings of the Royal Society, London B, 169: 298-310.

Huenneke L. F. 1991. Ecological implications of genetic variation in plant populations. In: D. A. Falk & K. E. Holsinger. (Eds.). Genetics and Conservation of Rare

Plants. Pag. 31-44. Oxford University Press, New York.

Lutz E., Schneller J. J. & Holderegger R. 2000. Understanding population history for conservation purposes: population genetics of *Saxifraga aizoides* (Saxifragaceae) in the lowlands and lower mountains north of the Alps. American Journal of Botany, 87 (4): 583-590.

Miller M. P. 1997. Tools for population genetic analyses (TFPGA) 1.3: A windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by author.

Nei M. 1972. Genetic distance between populations. American Naturalist, 106: 283–292.

Nei M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nybom H. & Bartish I. V. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtaneid with RAPD markers in plants. Perspectives in Plant Ecology, Evolution and Systematic, 3/2: 93-114.

Passalacqua N. G. & Bernardo L. 1997. Flora relitta di altitudine dell'Appennino meridionale: quale origine? Biogeographia, 19: 105-117.

Pignatti E., Pignatti S., Nimis P. & Avanzini A. 1980. La vegetazione ad arbusti spinosi emisferici. Contributo alla interpretazione delle fasce di vegetazione delle alte montagne dell'Italia mediterranea. C.N.R. AQ/1/79, Roma.

Pignatti S. 1982. Flora d'Italia. Vol. 3: 14-16. Edagricole, Bologna.

Schaal B. A., Hayworth D. A., Olsen K. M., Rauscer J. T. & Smith W. A. 1998. Phylogeographic studies in plants: problems and perspectives. Molecular Ecology, 7: 465-474.

Shaw C. R. & Prasad R. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochemical Genetics, 4: 297-320.

Sneath P. H. A. & Sokal R. R. 1973. Numerical taxonomy. Freeman W. H. San Francisco.

Sokal R. R. & Rohlf F. J. 1995. Biometry. W. H. Freeman, New York.

Soltis D. E., Haufler C. H., Darrow D.C. & Gastony G. J. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. American Fern Journal, 73 (1): 9-27.

Stehlik I. 2000. Nunataks and peripheral refugia for alpine plants during quaternary glaciation in the middle part of the Alps. Botanica Helvetica, 110: 25-30.

Stehlik I., Schneller J. J. & Bechmann K. 2001. Resistance or emigration: response of the high-alpine plant *Eritrichium nanum* (L.) Gaudin to the ice age within the Central Alps. Molecular Ecology, 10: 357-370.

Sun G. L., Salomon B. & von Bothmer R. 1999. Genetic diversity in *Elymus caninus* as revealed by isozyme, RAPD and microsatellites markers. Genome, 42: 1-12.

Thompson J. D. 1999. Population differentation in Mediterranean plants: insights into colonization history and evolution and conservation of endemic species. Heredity, 82: 229-236.

Tutin T. G. 1976. *Adenostyles* Cass. In: Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. & Webb, D. A. (Eds.). Flora Europea. Pag 189. Cambridge University Press, Cambridge.

Vrijenhoek R. C. 1987. Population genetics and conservation. In: D. Western & M. C. Pearl. (Eds.). Conservation for the

21st Century. Pag. 89-98. Oxford University Press, New York.

WAGENITZ G. 1983. Die Gattung *Adenostyles* Cass. (Compositae-Senecioneae). Phyton (Austria), 23 (1): 141-159.

WEEDEN N. F. & WENDEL J. F. 1990. Genetics of plant isozymes. In: Soltis D. E., Soltis P. S. (Eds.). Isozymes in plant biology. Dudley T. R., Ph. D., General Editor. Pag. 46-72. Chapman and Hall, London.

WEIR B. & COCKERHAM C. C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, 38: 1358-1370.

WENDEL J. F. & WEEDEN N. F. 1990. Visualization and interpretation of plant isozymes. In: Soltis D. E., Soltis P. S. (Eds.). Isozymes in plant biology. Dudley T. R., Ph. D., General Editor. Pag. 5-45. Chapman and Hall, London.

WILLIAMS J. G. K., KUBELIK A. R., LIVAK K. J., RAFALSKI J. A. & TINGEY S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18: 6531-6535.

WILLIS K. J. 1996. Where did all the flowers go? The fate of temperate European flora during glacial periods. Endeavour, 20 (3): 110-114.

WOLFE K. & MORGAN-RICHARDS M. 1998. PCR markers distinguish *Plantago major* subspecies. Theoretical and Applied Genetics, 96: 282-286.

WRIGHT G. 1965. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution, 19: 395-420.

YEH F. C., YANG R. C., BOYLE T. B. J., YE Z. H. & MAO J. X. 1997. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada.

ZHANG L., COMES H. P. & KADEREIT J. 2001. Phylogeny and quaternary history of the European montane/alpine endemic *Soldanella* (Primulaceae) based on ITS and AFLP variation. American Journal of Botany, 88 (12): 2331-2345.

Finito di stampare nell'ottobre 2003