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## Genetic variation patterns in some groups of *Centaurea* (Asteraceae) in Italy

### Abstract

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In the current work the data of the isozyme analysis of all the twenty studied Italian *Centaurea* taxa were compared. They belong to *Centaurea parlatoris*, *C. cineraria*, *C. tenorei* and *C. jacea* groups. The following parameters of the genetic variability were considered: the total number of the alleles, the mean number of alleles per locus (A), the mean percentage of polymorphic loci (P95), the expected heterozygosity (He) that is a measure of the intra-population diversity, the Wright's fixation index (F) and inbreeding coefficient ( $F_{IS}$ ) that are measures for the deviation from the Hardy-Weinberg equilibrium.

The present study contributes to insight some aspects of the genetic diversity related to the distribution range (endemics or widespread taxa) of the studied *Centaurea* species. The mean proportion of polymorphic loci and the mean number of alleles per locus (A) in the endemic species are comparable with the widespread ones, while the intra-population diversity (He) results about 40% higher in the populations of the widespread taxa than the endemics. The loci with a major role for the processes of differentiation and evolution of the genus *Centaurea* in South Italy and Sicily are: *Mdh-1*, *Pgi-2* and *Idh-1*.

Italian *Centaurea* species with the highest genetic diversity usually inhabit extreme habitats (rocky, sandy places, etc.) and localities with significant human impact or the borderline of their area of distribution. Sicily could be considered a centre of differentiation of the endemic groups of *C. cineraria* and *C. parlatoris* which show relatively high intra-population variability.

### Introduction

*Centaurea* L. is one of the largest and taxonomically intricate genera of Asteraceae distributed predominantly in the Old World (Dittrich 1977; Bremer 1994; Wagenitz & Hellwig 1996; Greuter & al. 2001; Greuter 2003). The East Anatolia and the Transcaucasus are the primary centres of origin and diversity while the Mediterranean area and the Balcan Peninsula are the secondary centres (Wagenitz 1975; Wagenitz & Hellwig 1996; Routsis & Georgiadis 1999). Depending on the adopted classification, genus *Centaurea* includes between 200 and 700 species (Bremer 1994; Wagenitz & Hellwig 1996; Hellwig 2004). The degree of differentiation among the separated taxa is, in some cases, unclear, because of their high morphological variation within and among the populations. Recently, the increased interest in the phylogeny and evolution of characters of *Centaurea*, have led to examine different aspects including floral and achene micro-morphology, pollen morphology, karyology, DNA sequences, genome size, isozyme analysis etc. (Garcia-Jacas &

Susanna 1992; Susanna & al. 1995; Garcia-Jacas & al. 1996, 2000, 2001; Wagenitz & Hellwig 1996; Routsis & Georgiadis 1999; Font & al. 2002; Palermo & al. 2002; Bancheva & Raimondo 2003; Bancheva & Greilhuber 2006; Bancheva & al. 2006; Guarino & Rampone 2006; Bancheva & al. 2011). Chromosome basic numbers, pollen structure and DNA sequences are the most reliable characters. The isozyme method is also very useful for the detection of similarities or differences among plant populations and taxa, due to the strong relationships between allozyme alleles and environmental characteristics (Prentice & Cramer 1990; Prentice & al. 1995).

The genus *Centaurea* is represented in Italy by about 130 taxa (species and subspecies) (Conti & al. 2005) and has a very complicated taxonomy. It comprises annual to perennial herbs, rarely dwarf shrubs with undivided to pinnatisect leaves. Capitula are solitary or in groups of 2-3 at the apex of the branches. Involucre is from cylindrical to spheroidal, bracts are often with a fimbriate or spiny appendage. Inner florets are hermaphrodite, outer – sterile. Achenes are somewhat laterally compressed, with lateral hilum, often with elaiosome. Pappus is constituted by several series of unequal bristles, sometimes absent (Dostál 1976). In the Italian flora almost the 48% of the *Centaurea* taxa have a very restricted area of distribution and/or are endemics.

The present study deals with genetic variation patterns of 20 taxa of genus *Centaurea*, belonging to 4 groups - *C. parlatoris*, *C. cineraria*, *C. tenorei* and *C. jacea*. In the first 3 groups very close relatives are included, while the last group is composed by taxa of Wagenitz's *Jacea* pollen type (1955), but belonging to two different subgenera – *Seridia* (Juss.) Czerep. and *Solstitiaria* (Hill.) Dobrocz. (Dostál 1976).

The aims of the current work are: 1. to compare the genetic diversity of all the studied populations of genus *Centaurea* with Italian origin, using isozymes; 2. to assess the genetic diversity patterns of the close relatives and the taxa that have undergone a long term evolution; 3. to insight some aspects of the genetic diversity models of both the endemic species and the taxa with wide distribution.

## Material and methods

In the current work the data of the isozyme analyses of all the twenty studied Italian *Centaurea* taxa were compared. They belong to *Centaurea parlatoris*, *C. cineraria*, *C. tenorei* and *C. jacea* groups (Palermo & al. 2002; Bancheva & al. 2006, 2009, 2011). The nomenclature of the studied taxa and their localities in Sicily and Southern Italy are listed in Table 1 and Fig. 1. Seventeen species are endemics for Sicily or Italy (belonging to *C. parlatoris*, *C. cineraria*, *C. tenorei* groups), whereas three have a large distribution (*C. jacea* gr.).

The following parameters of the genetic variability were considered: the total number of the alleles, the mean number of alleles per locus ( $A$ ), the mean percentage of polymorphic loci ( $P_{95}$ ), the expected heterozygosity ( $H_e$ ) that is a measure of the intra-population diversity (Nei 1973), the Wright's fixation index ( $F$ ) (1951) and inbreeding coefficient ( $F_{IS}$ ) that are measures for the deviation from the Hardy-Weinberg equilibrium. Wright's  $F$ -statistics (1951) was also employed to analyse genetic structure. These measures represent the relative excess of homozygotes or heterozygotes compared with panmictic expect-

Table 1. List of the Italian *Centaurea* taxa investigated in the study.

Symbol	Taxa	Locality	Distribution
△	<i>Centaurea tenorei</i> Guss. ex <i>Lacaita</i> f. <i>tenorei</i>	Southern Italy, Campania, Lattari Mts M. S. Angelo (Salerno)	Sorrentine Peninsula (Endemic)
	<i>C. tenorei</i> f. <i>maritima</i> Lacaita	Southern Italy, Campania, Peninsula Sorrentina (Salerno)	Coasts of Amalfi (Endemic)
	<i>C. tenorei</i> f. <i>montaltensis</i> Lacaita	Southern Italy, Campania, M. Avvocata, Maiori village Salerno)	Coasts of Amalfi (Endemic)
	<i>C. scannensis</i> (Anzalone) Pign.	Central Italy, Abruzzo, Anversa degli Abruzzi village (L'Aquila)	Abruzzo Region (Endemic)
	<i>C. ambigua</i> Guss.	Abruzzo, Cinquemiglia plane-Roccaraso (L'Aquila)	Apuane Alps and Appennino Tosco-Emiliano locality (Endemic)
◆	<i>C. parlatoris</i> Heldr. var. <i>virescens</i> Guss.	Sicily, M. Occhio, Montelepre village 500 a.s.l. (Palermo)	Sicily, Southern Italy, (Endemic)
	<i>C. parlatoris</i> Heldr. var. <i>parlatoris</i>	Sicily, Madonie Mts, Vallone Madonna degli Angeli, 1386 m a.s.l. (Palermo)	Sicily (Endemic)
		Sicily, Madonie Mts, Viaduct Faguara, 1592 m a.s.l. (Palermo)	Sicily (Endemic)
		Sicily, Madonie Mts, Isnello village, in the cliffs, 583 m a.s.l. (Palermo)	Sicily (Endemic)
		Sicily, San Martino delle Scale, 700 m a.s.l. (Palermo)	
	<i>C. sicana</i> Raimondo & Spadaro	Sicily, Monte Cammarata, above the refuge (Agrigento)	Sicily (Endemic)
	<i>C. giardiniae</i> Raimondo & Spadaro	Sicily, Etna, above Nicolosi village, 822 m alt. (Catania)	Sicily (Endemic)
○	<i>C. cineraria</i> L. subsp. <i>cineraria</i>	Southern Italy, Campania, Vietri village, 10 m a.s.l. (Salerno)	Southern Italy (Endemic)
		Southern Italy, Campania, Minori village, 10 m a.s.l. (Salerno)	
	<i>C. busambarensis</i> Guss.	Sicily, Rocca Busambra of Ficuzza, 1500 m a.s.l. (Palermo)	Sicily (Endemic)
	<i>C. ucraiae</i> Lacaita subsp. <i>ucraiae</i>	Sicily, Monte Passo del Lupo, 50 m a.s.l. (Trapani)	Sicily (Endemic)
		Sicily, Monte Inici, 100 m a.s.l. (Trapani)	
		Sicily, Monte Cofano (Trapani)	
	<i>C. ucraiae</i> subsp. <i>umbrosa</i> (Lacaita) Cela Renzoni & Viegi	Sicily, Monte Pellegrino, 80 m. a.s.l. (Palermo)	Sicily (Endemic)
	<i>C. todaroi</i> Lacaita	Sicily, next to motorway Palermo-Catania, Bagheria, 90 m a.s.l. (Palermo)	Sicily (Endemic)
		Sicily, Monte Catalfano, Mongerbino area, 150 m a.s.l. (Palermo)	
	<i>Centaurea erycina</i> Raimondo & Bancheva	Sicily, Castello di Venere - Erice village, 600 m a.s.l. (Trapani)	Sicily (Endemic)
	<i>C. saccensis</i> Raimondo, Bancheva & Ilardi	Sicily, near Sciacca village, 150 m a.s.l. (Agrigento)	Sicily (Endemic)
○	<i>C. solstitialis</i> subsp. <i>schowii</i>	Sicily, near Contessa Entellina (Palermo)	Sicily, Calabria, Basilicata Regions (Endemic)
	<i>C. sphaerocephala</i> L.	Sicily, Biviere di Gela, (Caltanissetta)	SW-Steno-Mediterranean
	<i>C. napifolia</i> L.	Sicily, San Fratello village (Messina)	SW-Steno-Mediterranean
	<i>C. nicaeensis</i> All.	Sicily, Piana degli Albanesi (Palermo)	SW-Steno-Mediterranean

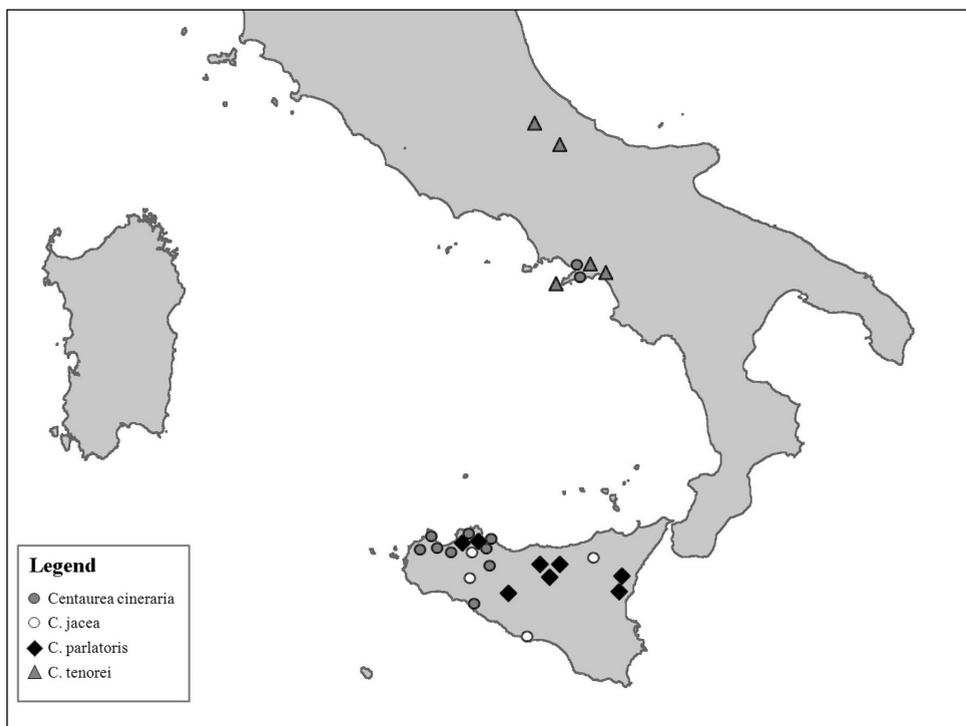


Fig. 1. Distribution map of studied Italian *Centaurea* taxa.

tations relative to all populations ( $F_{IT}$ ), within populations ( $F_{IS}$ ) and among populations ( $F_{ST}$ ). The last coefficient estimates relative population differentiation. The gene flow ( $Nm$ ) was calculated as  $Nm = (1 - F_{ST}) / 4 F_{ST}$ . Genetic relationships among populations were calculated by computing the standard genetic distance ( $D$ ) (Nei 1972, 1978). Besides the number of the revealed loci and the respective allele forms for each enzyme systems, as well as the genetic distances ( $D$ ) (Nei 1978) between the taxa from each group with others groups have been compared.

## Results

### *Allele frequencies*

The total number of the alleles determined in the different groups ranges from 11 in *C. tenorei* gr. (in 6 loci) to 23 in *C. parlatoris* gr. (in 7 loci) (Table 2). Besides, in the last group we found a high number of alleles at *Mdh-1* locus (6 alternative forms) and at *Pgi-2* locus (4 alternative forms) (Table 3). The locus *Pgm-1* results monomorphic in the populations of *C. cineraria* gr. and *C. parlatoris* gr., whereas it shows three allelic forms in the populations of *C. jacea* gr. The locus *6Pgd-1*, which is also monomorphic in *C. cineraria* gr., presents 3 allelic forms in *C. parlatoris* gr. (Table 3).

The mean number of alleles per locus (A) is comparable in all the studied groups and ranges from 1.46 (*C. tenorei* gr.) to 1.75 (*C. cineraria* gr.) (Table 2).

The mean proportion of polymorphic loci (using the 0.95 criterion) varies from 41% (*C. cineraria* gr.) to 53.6% (*C. jacea* gr.). The lowest polymorphism values (P95) were registered in the populations of *C. ucraiae* Lacaita subsp. *ucraiae* (25%), *C. napifolia* L. and *C. parlatoris* Heldr. from Isnello, Sicily (28.6%), whereas the populations of *C. tenorei* f. *maritima* Lacaita (83.3%), *C. nicaeensis* All. e *C. sphaerocephala* L. (71.4%) showed the highest values (Palermo & al. 2002; Bancheva & al. 2006, 2009, 2011).

In *C. parlatoris* gr. 7 unique (exclusive) alleles and 5 rare ones were determined, while in *C. cineraria* gr. 6 rare alleles and 2 unique ones are registered. In *C. jacea* gr. there are two unique alleles, but no rare alleles were found (Table 2).

#### *Intra-population variability (He)*

The mean values of  $H_e$  range between 0.16 (*C. tenorei* gr.) and 0.28 (*C. jacea* gr.). The high values of the genetic variability are usually associated with a large ecological plasticity. In the populations of *C. tenorei* f. *maritima* and *C. tenorei* f. *montaltensis* Lacaita the lowest values of  $H_e$  (0.08 and 0.14) were registered. The populations of *C. sphaerocephala* and *C. nicaeensis*, however, showed relatively high values of  $H_e$  0.359 and 0.276, respectively (Palermo & al. 2002; Bancheva & al., 2006, 2009, 2011). The endemic species, generally, showed lower heterozygosity values than the widespread ones, but some populations, situated in localities with high human impact, present very high  $H_e$  values.

#### *Inbreeding coefficient ( $F_{IS}$ )*

In the majority of *Centaurea* taxa, the values of the inbreeding coefficient result negative. In the populations of *C. cineraria* gr. and *C. parlatoris* gr. the value of this coefficient results close to zero while it is negative in the *C. jacea* populations. It may indicate that

Table 2. Comparison between the parameters of genetic diversity in the examined *Centaurea cineraria*, *C. jacea*, *C. parlatoris* and *C. tenorei* groups.

	<i>C. cineraria</i>	<i>C. jacea</i>	<i>C. parlatoris</i>	<i>C. tenorei</i>
<b>Number of loci</b>	8	7	7	6
<b>Total alleles</b>	19	17	23	11
<b>Unique alleles</b>	2	2	7	no data
<b>Rare alleles</b>	6	-	5	no data
<b>P95</b>	41	53.6	46.44	49.7
<b>A</b>	1.75	1.68	1.74	1.46
<b>He</b>	0.18	0.28	0.19	0.16
<b>F<sub>IS</sub></b>	-0.051	-0.43	-0.072	0.42
<b>F<sub>ST</sub></b>	0.24	0.43	0.27	0.34
<b>Nm</b>	0.792	0.331	0.676	0.485
<b>Dmin</b>	0.002	0.244	0	0.079
<b>Dmax</b>	0.158	0.638	0.211	0.318
<b>Dmean</b>	0.077	0.437	0.097	0.197

Table 3. Number of alleles at each locus in the investigated *Centaurea cineraria*, *C. jacea* and *C. parlatoris* groups

System	Locus	<i>C. cineraria</i>	<i>C. jacea</i>	<i>C. parlatoris</i>
IDH	<i>Idh-1</i>	3	2	3
	<i>Idh-2</i>	-	2	-
MDH	<i>Mdh-1</i>	3	3	6
	<i>Mdh-2</i>	2	-	-
6PGD	<i>6Pgd-1</i>	1	no data	3
	<i>6Pgd-2</i>	2	no data	3
PGI	<i>Pgi-2</i>	3	3	4
PGM	<i>Pgm-1</i>	1	3	1
	<i>Pgm-2</i>	3	3	3

the populations are outcrossers and strongly auto-incompatible. Only *C. tenorei* f. *maritima* has a very high value of  $F_{IS}$  (0.73) that indicates insufficiency of heterozygotes among the population and high self-compatibility of the taxon. This trait could have been acquired as an evolutionary consequence of polyploidisation (Palermo & al. 2002).

#### *Inter-population variability coefficient ( $F_{ST}$ ) and genetic distances*

The inter-population variability coefficient in Italian *Centaurea* taxa ranges from 0.24 (*C. cineraria* gr.) to 0.43 (*C. jacea* gr.). The low values found in *C. cineraria* gr. and *C. parlatoris* gr. (0.27) proved a relatively recent differentiation of the taxa, while the high  $F_{ST}$  values of *C. jacea* gr. may indicate a long period of separation of the included species. These data are very well supported also by the values of the genetic distances among the studied *Centaurea* groups. The lowest values were found in *C. cineraria* gr. (0.077) and *C. parlatoris* gr. (0.097); *C. tenorei* showed the intermediated value (0.197), while the highest values of the genetic distance were identified in *C. jacea* gr. (mean value 0.437).

#### *Gene flow ( $Nm$ )*

The values of the gene flow are less than 1 for all the Italian *Centaurea* populations: from 0.331 in *C. jacea* gr. to 0.792 in *C. cineraria* gr.

## Discussion and Conclusions

The analysis of enzyme polymorphisms has proved to be useful in assessing the levels and the distribution of genetic variation within and among populations of single or closely related species and it may be an effective tool in the study of their taxonomy, evolution and conservation (Hamrick 1990).

The present study contributes to insight some aspects of the genetic diversity related to the distribution range of the studied *Centaurea* species: endemics or widespread taxa. Information on genetic diversity patterns also reveals the evolutionary and demographic history of the taxa. All the species of *C. tenorei* gr., *C. parlatoris* gr. and *C. cineraria* gr.

are endemics to Southern Italy or Sicily and some of them have been recently described (Raimondo & Bancheva 2004; Raimondo & al. 2004; Raimondo & Spadaro 2006, 2008). The genetic variation pattern shows that the taxa into these groups are very close relatives and a recently initiated phylogenetic divergence could be presumed (Palermo & al. 2002; Bancheva & al. 2006, 2009, 2011).

Several authors showed that the geographic range of species could be a good predictor of genetic diversity of natural populations, where narrowly distributed or endemic species attain lower genetic diversity levels than their widespread congeners (Karron 1987; Hamrick & Godt 1989, 1996; Gitzendanner & Soltis 2000; Cole 2003; Mateu-Andrés 2004; Orellana & al. 2009). Widespread species, often consisting of historically larger and more continuous populations, maintain higher polymorphism than endemics and are less affected by drift, which tends to erode genetic variation in more geographically restricted species (Premoli & al. 2001).

Other studies, on the contrary, show that also narrow endemics could have high genetic diversity parameters (Cardoso & al. 1998; Delgado & al. 1999; Gonzàles-Astora & Castillo-Campos 2004; Torres-Diaz & al. 2007).

Our observations on *Centaurea* revealed that the mean proportion of polymorphic loci (using the 0.95 criterion) and the mean number of alleles per locus (A) of the populations of the endemic species are comparable with the populations of the widespread ones, while the intra-population diversity ( $H_e$ ) results about 40% higher in the populations of the taxa with large areal than in the endemics. This last index (mean expected heterozygosity) is thought to be the most effective one for comparing genetic variation (Nei 1987). Moreover, the widespread *Centaurea* species have not rare alleles and the alleles are uniformly distributed.

The loci with a major importance for the processes of differentiation and evolution of genus *Centaurea* in Southern Italy and Sicily are: *Mdh-1*, *Pgi-2* and *Idh-1*.

In our study on *Centaurea* species the mean value of the genetic distances (D) of these groups that include close relatives ranges between 0.077 and 0.197. However, this index reaches the value of 0.638 (D max) in the group of the very well morphologically distinguished taxa due to long divergence processes (*Jacea* gr.), whereas it reaches the minimal value in the populations with recent origin, whose taxa have marked morphological and ecological discrepancies but their genetic diversity is still not significant (*C. cineraria* and *C. parlatoris* groups) (Table 2). According to Crawford (1985, 1990) plant speciation may occur without divergence in allozymes, and in these instances all available data suggest a rapid process (quantum speciation according to the general concept of Grant 1981).

The results prove that the genetic diversity is relatively high, not only within the single populations, but also among the populations of the species. Most of the *Centaurea* species are outcrossers and maintain high level of heterozygosity ( $F_{IS} = -0.43$ ). According to the over-dominant hypothesis, the fitness of populations should generally increase with the number of heterozygous loci. These populations could better adapt themselves to extreme ecological conditions. Heterozygous individuals often exhibit higher variability, greater developmental stability, and higher growth rates than homozygous ones (Seager & Ayala 1982; Carson 1987; Mitton 1990).

Only in the population of *C. tenorei* f. *maritima* there is an excess of homozygotes – index  $F_{IS}$  has a relatively high value. This taxon is tetraploid, very probably - self-com-

patible and subject to Wahlund effect (1928). According to Cole (2003) the species monomorphic for isozymes are predominantly endemic and self-fertile.

In widespread and rare plants  $F_{IS}$  and  $F_{ST}$  do not differ significantly, reflecting both the similarity of breeding system in congeneric species and the disparate ranges often sampled for rare and common species (Cole 2003). Our data show that the taxa from *C. jacea* gr. (widespread) have very high values of both  $F_{IS}$  and  $F_{ST}$ .  $F_{ST}$  indicates the level of genetic diversity among populations. In our study we grouped as “*C. jacea* gr.” 4 species with *Jacea* pollen type (*sensu* Wagenitz 1955) and belonging to 2 subgenera of *Centaurea*. The genetic distances among all the examined taxa are clearly expressed and they reflect Dostál’s subdivision (1976). It seems that the plant speciation of this group has occurred in remote time. The genetic distances between taxa of subgenus level are significantly higher than the genetic distances between close relative species (Bancheva & al. 2006, 2009, 2011).

Regarding gene flow, in all the studied groups the value of the index  $Nm$  is lower than 1. When the gene flow has a high value, the populations tend to remain more or less uniform, whereas, when the gene flow has a low value the populations tend to diverge. The gene flow has relatively low values in *C. tenorei* and *C. jacea* groups. In the first case it is caused by the inbreeding in the species, in the second case the species are morphologically and genetically well differentiated due to a long term evolution.

The taxa of *C. cineraria* group exhibit the highest value of the coefficient of gene flow ( $Nm=0.792$ ). It could be considered indicative for a recently initiated phylogenetic divergence. It is very likely that in this group some gene flow still exists .

The values of the coefficient  $F_{ST}$  (0.17) and  $Nm$  (1.17) in five populations comprising *C. parlatoris* subsp. *parlatoris* and *C. parlatoris* var. *virescens* Guss. show an elevated similarity among these populations. At the same time they are relatively well separated by the two recently described species – *C. sicana* Raimondo & Spadaro and *C. giardiniae* Raimondo & Spadaro (Raimondo & Spadaro 2006, 2008) belonging to the same group: the values of  $F_{ST}$  and  $Nm$ , in fact, resulted 0.27 and 0.676 respectively, when all the taxa of *C. parlatoris* gr. were analyzed.

In conclusion, we can summarize that the Italian *Centaurea* species showing the highest genetic diversity usually inhabit extreme habitats (rocky, sandy places, etc.) and localities with significant human impact or they are distributed on the borderline of their own area of distribution. Our study on these four *Centaurea* groups revealed that the values of the genetic diversity are more closely related to the ecological conditions (environmental pressure) than to the size of the area of distribution. The results of the distribution of the genetic diversity in the studied groups show that Sicily could be considered as a centre of differentiation of the endemic taxa related to *C. cineraria* and *C. parlatoris* that have relatively high intra-population variability and probably still diverge.

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