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Genetic diversity and local population structure in *Ambrosina bassii* (Araceae, Ambrosineae), a Mediterranean relict species

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ABSTRACT

The effects of habitat fragmentation on the genetic structure of *Ambrosina bassii* are analyzed. The species, whose reproductive biology is mostly unknown, is the only representative of its genus and tribe and it is endemic to the central Mediterranean area. The selected study area was the island of Sicily, in which wild populations show a wide morphological variability and ecological amplitude. Patterns of within- and among-population genetic diversity in eleven Sicilian populations, occurring in six disjunct areas, were examined by means of allozyme electrophoresis. High levels of genetic diversity were found as shown by the mean expected heterozygosity ($H_e = 0.263$), the percentage of polymorphic loci ($P_{95} = 65.3$), the mean number of alleles per locus ($A = 2.0$). Genetic differentiation between populations was relatively low (mean $F_{ST} = 0.091$ and $Nm = 1.98$). A very weak correlation exists between genetic distances and geographic distances between populations. Despite its restricted and fragmented geographical range, *A. bassii* showed (i) high levels of genetic diversity, mainly within populations; (ii) no genetic differentiation between populations and morphotypes.

1. Introduction

The patterns of genetic variation within and among populations are of interest to several fields in plant biology including population genetics, systematics, and conservation (Levsen et al., 2008). Many factors interact to determine genetic structure within populations including adult density, mating systems, colonization history, natural selection, and mechanism of gene dispersal (Jones et al., 2006).

Several studies have been carried out in order to look for genetic effects in plants of recent, anthropogenic, habitat fragmentation (e.g. Ellstrand and Elam, 1993; Honnay et al., 2006). Since the responses to fragmentation may vary depending on habitat or life history characteristics (Young et al., 1993), more studies on genetic consequences of fragmentation need to be conducted.

We have chosen to focus on *Ambrosina bassii*, a poorly known species representing an isolated evolutionary lineage within the Araceae, and occurring today in disjunct areas of the Central-Western Mediterranean Basin. In particular, we focused on the effects of local anthropogenic fragmentation of populations occurring in the island of Sicily on the genetic structure of the species. Sicily was chosen because it is an isolated circumscribed territory, in which wild populations show a wide morphological variability and ecological amplitude.

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In order to estimate levels of intraspecific genetic variation we used allozyme markers, which proved to be an invaluable tool to this purpose (Levsen et al., 2008; Jones et al., 2006) but also to verify the taxonomic significance of some morphotypes (Salmaki et al., 2009).

2. Material and methods

2.1. The species

A. bassii L., a perennial diploid species with 2n = 22 (Vignoli, 1939; Scrugli and Bocchieri, 1976), is the only representative of its genus; on the basis of this basic number $x = 11$ and of the large (8–12 μm) completely acrocentric chromosomes, Petersen (1989) pointed out the isolated position of *Ambrosina* within the tribe Areae, proposing its exclusion from the subfamily Aroideae. Recently, Mayo et al. (1997) confirmed the isolated position of the genus placing it in the monotypic tribe Ambrosineae, within the subfamily Aroideae.

Ambrosina, together with the genus *Arisarum* (included in another monotypic tribe) (see Barabé et al., 2004; Cabrera et al., 2008), was the first member of Araceae to colonize the Mediterranean area during the Late Cretaceous (around 82.7 Ma b.p.) and might have been part of an ancestral subtropical flora that was likely widespread throughout the Tethyan region (Mansion et al., 2008).

The range of *A. bassii* (that corresponds with the range of the genus and the tribe too) is limited to a few areas of the Central-Western Mediterranean, including Southern Italy, Sicily, Sardinia, Corsica, Tunisia and Algeria. Fig. 1, showing the distribution area of the species, was constructed on the basis of the figure of Mayo et al. (1997), completed with data concerning Tunisia (Bonnet and Barratte, 1896; Angelo Troia, pers. obs.) and Southern Italy (Conti et al., 2005). An interesting comparison can be made between this distribution and other ones, such as those of some ancient sections of the genus *Cytisus* (Cristofolini and Troia, 2006).

A. bassii is a small herb with a rhizomatous tuber as a stem. The plant loses its leaves during the hot dry summer season. The inflorescence is probably unique within the entire family: solitary, lying horizontally on the ground, it bears a single female flower in an upper (ventral) chamber, and several male flowers in an internal (dorsal) chamber; the upper chamber is open to the external environment, and communicates with the internal chamber by a small hole. This arrangement into two separate chambers is an "extreme case" of "elaborate modification" of the spathe, found in few members of the family such as *Cryptocoryneae*, *Pistia* and *Pinellia* (Mayo et al., 1997). Flowering time (November–March) is unusual for Mediterranean region but, interestingly, occurs in other mediterranean endemics of Araceae, including *Arum pictum* or *Biarum dispar* (Mayo et al., 1997; Mansion et al., 2008). Fruit-set appears extremely rare (Killian, 1929; Troia et al., 2009).

Pollination syndromes in *A. bassii* remain largely unknown. Available information mentioned only a few visitors (e.g. insects or other small invertebrates), but the identity of potential pollinators is still a matter of speculation. Killian (1929), Vignoli (1939) and Mayo et al. (1997) hypothesize that the pollinators are extinct. Killian (1929), although emphasizing the possibility of self-pollination, observed poor fruit sets in wild Algerian populations, a fact we confirmed in Sicily. Overall prevailing vegetative reproduction syndromes can be hypothesized.

2.2. Study sites and sampling

In Sicily, *Ambrosina* is widespread although not common, growing on different geological substrates from 0 to ca. 700 m a.s.l., mainly in open stony habitats but also in the understory of maquis and woodlands.

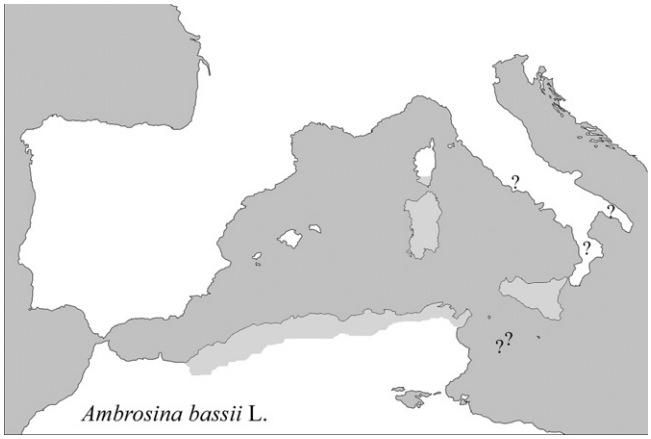

Fig. 1. Distribution area of *Ambrosina bassii* (from Mayo et al., 1997, adapted and modified).

During the 2007–2008 winter, eleven populations of *A. bassii* were sampled in Sicily (see Table 1 and Fig. 6), to represent different ecological/geographical sites. About 15 distinct individuals were collected for each population. In general, population size varied from several tens to hundreds of individuals.

In this work we had the opportunity to verify, from a genetic point of view, the value and significance of the remarkable leaf morphological variability occurring in Sicilian populations. This variability led in the past to describe several taxa as distinct species (*Ambrosina maculata* Ucria, *Ambrosina reticulata* Tin.) or variety (*A. bassii* var. *angustifolia* Guss.) (Figs. 2–5). The most recent Floras and monographies ignore these taxa (Pignatti, 1982; Mayo et al., 1997; Conti et al., 2005) or consider them at a varietal rank (Giardina et al., 2007).

As it is shown in Table 1, sampled populations can be referred to *A. bassii* var. *bassii* (henceforth simply "*bassii*") and to the taxa described in Sicily between the XVIII and XIX Century, namely: *A. maculata*, *A. reticulata* and *A. bassii* var. *angustifolia* (henceforth simply "*maculata*", "*reticulata*" and "*angustifolia*", respectively). This last *taxon* is typical of *Chamaerops humilis* garrigues and open habitats in the SW part of the region (around the towns of Mazara del Vallo and Marsala, in the province of Trapani), but it was also found occasionally in a locality near Palermo (San Martino). "*Maculata*" and "*reticulata*" occur rarely and sparsely, so it was only possible to collect a few plants; for this reason, we decided to gather the "*maculata*" samples in 1 unit ("BES"). Voucher specimens are in the *Herbarium Mediterraneum* of Palermo (PAL).

2.3. Electrophoretic analyses

The following enzyme systems were examined: IDH – isocitrate dehydrogenase (E.C.1.1.1.42), MDH – malate dehydrogenase (E.C.1.1.1.37), 6PGD – 6-phosphogluconate dehydrogenase (E.C.1.1.1.44), MNR – menadione reductase (E.C. 1.6.99.2), PGI – phosphoglucoisomerase (E.C.5.3.1.9), PGM – phosphoglucomutase (E.C.2.7.5.1.). Concerning their quaternary structure, the last enzyme is monomer, menadione reductase is tetramer, the others are dimers (Weeden and Wendel, 1989).

Fresh young leaves, collected in the wild and transported to the laboratory, were crushed in a 200 μl buffer containing TrisHCl, pH 7.5, and 1% reduced glutathione. Crude extracts were absorbed on paper wicks and stored at −80 °C until use.

Table 1
Details of sampled populations of *Ambrosina bassii* (see also Fig. 6).

Taxon	Pop. code	Number of sampled individuals	Locality	Ecological parameters	Coll.
A. bassii '*bassii*'	A	14	S. Martino delle Scale (PA) 38°5′18.80″N 13°15′21.21″E	Alt. 550 m a.s.l.; Geological substr.: dolostones	30-01-2008 A.T.
A. bassii '*bassii*'	D	12	M. Gallo (PA) 38°12′37.41″N 13°18′27.66″E	Alt. 150 m a.s.l.; Geological substr.: limestones	01-02-2008 A.T.
A. bassii '*bassii*'	F	15	Mazara del Vallo (TP) 37°36′50.12″N 12°41′31.25″E	Alt. 60 m a.s.l.; Geological substr.: limestones	05-02-2008 A.T.
A. bassii '*bassii*'	I	15	Santa Ninfa (TP) 37°47′51.92″N 12°54′7.98″E	Alt. 520 m a.s.l.; Geological substr.: gypsum rocks	14-02-2008 A.T.
A. bassii '*bassii*'	M	14	Madonie-Collesano (PA) 37°55′24.56″N 13°57′7.96″E	Alt. 600 m a.s.l.; Geological substr.: limestones	19-02-2008 A.T.
A. bassii '*bassii*'	N	13	Ficuzza (PA) 37°53′18.70″N 13°24′52.17″E	Alt. 630 m a.s.l.; Geological substr.: quartzitic sandstones	22-02-2008 A.T.
A. bassii '*angustifolia*'	GR	13	Mazara del Vallo (TP) 37°36′50.12″N 12°41′31.25″E	Alt. 60 m a.s.l.; Geological substr.: limestones	28-02-2008 A.T.
A. bassii '*reticulata*'	K	2	Mazara del Vallo (TP) 37°36′50.12″N 12°41′31.25″E	Alt. 60 m a.s.l.; Geological substr.: limestones	05-02-2008 A.T.
A. bassii '*maculata*'	B	2	S. Martino delle Scale (PA) 38°5′18.80″N 13°15′21.21″E	Alt. 550 a.s.l.; Geological substr.: dolostones	30-01-2008 A.T.
A. bassii '*maculata*'	E	4	M. Gallo (PA) 38°12′37.41″N 13°18′27.66″E	Alt. 150 m a.s.l.; Geological substr.: limestones	01-02-2008 A.T.
A. bassii '*maculata*'	S	3	Mazara del Vallo (TP) 37°36′50.12″N 12°41′31.25″E	Alt. 60 m a.s.l.; Geological substr.: limestones	28-02-2008 A.T.

Fig. 2. *Ambrosina bassii* var. *bassii*.

Horizontal electrophoresis was performed under constant voltage at 4 °C on 11% starch gel according to Kephart (1990). Two buffer systems were used: Tris-citrate, pH 7.0 (Meizel and Markert, 1967) for MNR, PGI and PGM, and Morpholine-citrate, pH 6.1 (Clayton and Tretiak, 1972) for IDH, MDH and 6PGD. For the complete methodology see Geraci et al. (2004).

In a preliminary survey, some electrophoretic runs were also performed on cellulase acetate sheets, in order to test additional enzyme systems such as catalase (CAT), alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), and ribulose-bisphosphate carboxylase (RBC); for these systems not scorable data were obtained.

After migration, the gel slices were incubated in a staining solution specific for each enzyme according to Wendel and Stuber (1984) and Vallejos (1983). The loci and alleles were counted from the anode to the cathode.

Fig. 3. *Ambrosina bassii* var. *maculata*.

Fig. 4. *Ambrosina bassii* var. *reticulata.*

2.4. Data analysis

The allozyme frequencies, the mean number of alleles per locus (A), the mean percentage of polymorphic loci (P), observed (H_o) and expected (H_e) heterozygosity (according to the Hardy–Weinberg law) were calculated using BIOSYS-2 (Swofford and Selander, 2000). The Wright (1951) fixation index (F) was calculated as $F = 1 - H_o/H_e$. The Chi-square test was utilized to evaluate the significance of the deviation from the Hardy–Weinberg equilibrium.

Gene diversity, H, as described by Nei (1973), was calculated for each locus in each population.

The amount of genetic diversity at the gene pool level was estimated using Wright's (1965) F-statistics: F_{it}, F_{is} and F_{st}. F_{it} and F_{is} coefficients measure the excesses of homozygotes or heterozyogotes relative to the panmictic expectations within the entire sample and within populations, respectively. The F_{st} coefficient estimates the relative population differentiation. Genetic relationships between populations were calculated by computing the standard genetic distance (D) (Nei, 1972, 1978). Cluster analysis was performed by the UPGMA method using Nei's genetic identity measure.

Gene flow (Nm) among populations was estimated indirectly from the population genetic structure using Wright's (1951) equation as modified by Crow and Aoki (1984): $F_{ST} = 1/(4\,Nm\alpha + 1)$ where $\alpha = [n/(n-1)]^2$ and n is the number of populations.

To detect the geographical pattern of genetic differentiation, the significance correlation between the genetic distances and geographical distances (in km) between populations was tested.

Fig. 5. *Ambrosina bassii* var. *angustifolia.*

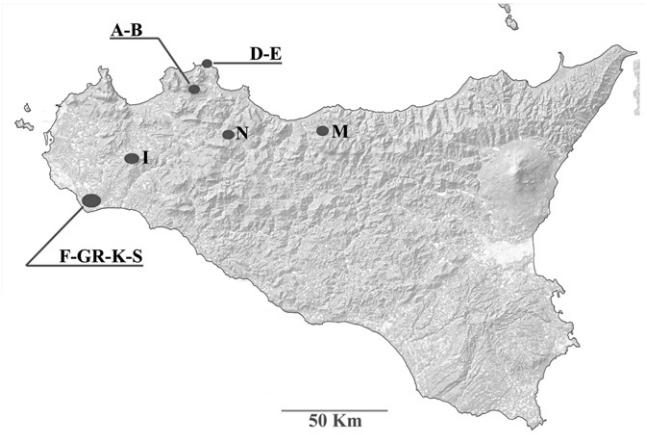

Fig. 6. Localization of the sampled populations (see also Table 1).

3. Results

3.1. Allele frequencies

An appreciable pattern of bands was obtained by means of 6 tested enzyme (PGM, PGI, MNR, 6PGD, MDH, IDH), and a total of 8 loci were analyzed. Twenty-five alleles were found in these loci and their frequencies for each population are reported in Table 2. The loci with the highest variability are *Pgi-2* showing six different alleles and *Pgm-1*, with four alleles; only *Mdh-1* locus resulted monomorphic, in all populations.

A great number (15) of rare alleles (whose frequency is $\leq$0.05) was found in different populations (Table 2) and 4 alleles were exclusive ("private") to some populations of "*bassii*": allele "c" at *Mnr-2* and allele "b" at *Pgi-2* in population "N" from Ficuzza, allele "c" at *Pgm-1* in population "F" from Mazara, allele "f" at *Pgi-2* in population "D" from M. Gallo. The last two

Table 2
Frequencies of allozymes at 8 loci detected in *Ambrosina* populations (codes of populations as in Table 1). Rare alleles (frequency $\leq$ 0.05) are in bold. Private alleles are marked with an asterisk (*).

Locus	Allele	A	D	F	I	M	N	K	BES	GR
Idh-1	a	0.071	0.083	0.267	0.233	0.607	0.269	1.000	0.222	
	b	0.929	0.917	0.733	0.733	0.393	0.731		0.722	1.000
	c				0.033				0.056	
Idh-2	a	0.929	1.000	1.000	0.733	0.750	1.000	1.000	1.000	1.000
	b	0.071			0.267	0.250				
Mdh-1	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mdh-2	a			0.233	**0.033**					0.077
	b	0.857	1.000	0.700	0.700	1.000	0.813	1.000	0.889	0.923
	c	0.143		0.067	0.267		0.188		0.111	
6Pgd-2	a	0.143	0.125	0.133	0.233	0.179	0.115		0.167	
	b	0.750	0.792	0.800	0.700	0.571	0.692	0.750	0.722	0.846
	c	0.107	0.083	0.067	0.067	0.250	0.192	0.250	0.111	0.154
Mnr-2	a	1.000	0.500	0.900	0.833	0.571	0.808	1.000	0.889	0.731
	b		0.500	0.100	0.167	0.429	0.115		0.111	0.269
	c						0.077*			
Pgi-2	a	**0.036**		0.067	0.132		**0.038**			**0.038**
	b						**0.038***			
	c	0.464	0.458	0.500	0.474	0.500	0.462	0.500	0.500	0.462
	d		**0.042**				**0.038**			
	e	0.500	0.458	0.433	0.395	0.500	0.423	0.500	0.500	0.500
	f		**0.042***							
Pgm-1	a	0.929	0.750	0.867	0.967	0.964	0.808	0.750	1.000	0.923
	b	**0.036**	0.250	0.067	**0.033**		0.192	0.250		
	c			**0.033***						0.077
	d	**0.036**		**0.033**		**0.036**				

Table 3
Allozyme genetic variability at 8 loci in all populations (for populations codes see Table 1): A = mean number of alleles per locus; P_{95}, P_{99} = proportion of polymorphic loci with the 0.95 and 0.99 criteria, respectively; H_o = observed heterozygosity; H_e = expected heterozygosity, F = Wright (1951) fixation index.

Population	A	P_{95}	P_{99}	Mean heterozygosity		F
				H_o	H_e	
A	2.1	75.0	75.0	0.241	0.205	−0.175
D	2.0	62.5	62.5	0.365	0.273	−0.337
F	2.4	75.0	75.0	0.250	0.279	0.103
I	2.4	75.0	87.5	0.318	0.340	0.064
M	1.9	62.5	75.0	0.321	0.323	0.006
N	2.4	75.0	75.0	0.335	0.314	−0.067
K	1.4	37.5	37.5	0.250	0.208	−0.201
BES	1.9	62.5	62.5	0.208	0.233	0.107
GR	1.8	62.5	62.5	0.250	0.192	−0.302
Mean	*2.0*	*65.28*	*68.06*	*0.282*	*0.263*	

alleles (together with allele "b" at *Pgi-2* in "N" population) were also rare; moreover, the allele 'd' at *Pgm-1* locus found in several populations ("A", "F", "M") and the allele 'd' at *Pgi-2* locus found in two populations ("F", "M") resulted rare.

3.2. Genetic variability

The mean proportion of polymorphism (P_{95} and P_{99}), the mean number of alleles per locus (A), the observed (H_o) and expected (H_e) heterozygosity, and the fixation index (F) for each population are shown in Table 3. The mean number of alleles per locus ranges from 1.4 in "*reticulata*" ("K") to 2.4 in "*bassii*" from Mazara del Vallo ("F"), Santa Ninfa ("I"), Ficuzza ("N"). The polymorphism is rather high (62.5–87.5%) in all examined populations except for "*reticulata*" (K) where the values resulted 37.5% (but only 2 individuals were sampled in this population). The difference between P_{95} and P_{99} in "I" and "M" populations is due to rare alleles.

The highest values of the mean heterozygosity index (H_e), which is a measure of intra-population variability, have been found in "*bassii*" "I", "M" and "N" populations (0.314–0.340). The lowest values were observed in "*angustifolia*" from "GR" population, Mazara del Vallo (0.192), and "*bassii*" from "A" population, San Martino delle Scale (0.205). In some examined populations, the observed heterozygosity was higher than expected, as revealed by the negative values of F.

Nei's gene diversity index (H) calculated for each polymorphic locus and for each population (Table 4), varies from 0.067 at the *Pgm-1* locus (population "I" from Santa Ninfa) to 0.667 at the *Mnr-2* and *Pgi-2* loci (population "D" from Monte Gallo and population "K" – "*reticulata*" – from Mazara, respectively). The latter locus exhibits the highest values in all populations because of its great number of alleles.

3.3. Diversity indices

F statistic was calculated per each polymorphic locus (Table 5). High values of F_{is} and F_{it} were scored in *Idh-1* and *Idh-2* loci showing a large excess of homozygous pattern, while negative values were found for the others except for *Mdh-2*. The mean value was ligthly less than 0 in F_{is} showing a small excess of heterozygotes.

The F_{st} values, which represent a measure of differentiation among all populations, resulted altogether rather low and comprised between 0.008 and 0.225, the mean value being 0.091. The loci mainly implicated in the differentiation among these populations are *Idh-1*, *Idh-2*.

3.4. Genetic relationships and gene flow

Table 6 shows the matrix of genetic distances and genetic identities. Values of genetic distance are ranged between 0 (between "BES" and "A", "F", "N", and between "N" and "F" populations) and 0.152. Nevertheless high values (higher than 0.1) occur only between "K" populations and other ones ("A", "GR", "I", "D").

Table 4
Nei's (1973) gene diversity for each population at each locus.

Locus/Pop	A	D	F	I	M	N	K	BES	GR
Idh-1	0.138	0.159	0.391	0.421	0.495	0.409	0	0.451	0
Idh-2	0.138	0	0	0.405	0.389	0	0	0	0
Mdh-1	0	0	0	0	0	0	0	0	0
Mdh-2	0.254	0	0.467	0.453	0	0.325	0	0.209	0.148
6Pgd-2	0.421	0.366	0.349	0.467	0.601	0.489	0.500	0.464	0.271
Mnr-2	0	0.667	0.186	0.287	0.508	0.342	0	0.209	0.409
Pgi-2	0.533	0.601	0.577	0.619	0.519	0.628	0.667	0.529	0.557
Pgm-1	0.140	0.391	0.251	0.067	0.071	0.323	0.500	0	0.148

Table 5
Summary of F-statistics at all polymorphic loci.

Locus	Fis	Fit	Fst
Idh-1	0.648	0.727	0.225
Idh-2	0.769	0.808	0.171
Mdh-2	0.299	0.380	0.116
6Pgd-2	−0.158	−0.118	0.035
Mnr-2	−0.044	0.096	0.100
Pgm-1	−0.183	−0.087	0.081
Pgi-2	−0.772	−0.757	0.008
Mean	**−0.085**	**0.014**	**0.091**

The dendrogram (Fig. 7) reveals one main group, embracing most of the populations (including var. *bassii*, *maculata*, *angustifolia*), with high values of genetic identity (0.98 or more); a second clade, including "M" ("*bassii*") and "K" ("*reticulata*") populations, is poorly distinguished from the former (genetic identity 0.92). The number of migrants per generation (Nm), a value that estimates the gene flow among these populations, was 1.98. Relating genetic distances between populations and geographical distances (in km), a positive correlation exists but it is very weak, as expressed by low values of R (0.4614) and R^2 (0.2129) (Fig. 8).

4. Discussion

The formation of spatial genetic structure, deriving from many interacting factors, is a crucial feature in plant evolutionary processes and population dynamics. Thus we have focused on the effects of the recent anthropogenic fragmentation of populations on a species with a distinctive mix of biological, biogeographic and phylogenetic features: in particular, the probably zoophilous but scarcely efficient sexual reproduction, the geographically restricted range with disjunct "sub-ranges", the isolated systematic position, respectively. The study area was a relatively large island (Sicily) in which discontinuous populations on several different geological substrates occur.

Our study revealed that the examined isolated populations show 1) high intra-population genetic diversity and 2) no clear inter-population divergence, as we expected on the basis of geographic discontinuity, ecological diversity, and a probable lack or scarsity of pollinators and limited dispersal capacity of the few seeds plants produce.

4.1. Intra-population diversity

Ambrosina maintains in Sicily a high level of genetic diversity in all populations as shown by the mean values of polymorphism, the number of alleles, the diversity intra-populations indices (Table 3). Fifteen rare alleles were found, and several ones (12) with a frequency less than 0.100 (Table 2); some of them were also private to single populations. The mean values of inbreeding coefficient (F_{is}) was negative (−0.08), also for 4 out of 7 polymorphic loci (Table 5). F values too, computed for single populations, resulted negative for 5 out of 9 examined populations (Table 3), revealing an excess of heterozygosity compared to the expected one. This could be referred to some outbreeding mechanisms.

The values of genetic diversity measures (Table 3) are high compared to the average of all plant species. For example, the mean H_e is 0.263, which is high compared to that of temperate-zone species (0.146), of species with sexual and asexual reproduction system (0.138), of long-lived herbaceous perennials (0.205) and of widespread species (0.202) (Hamrick and Godt, 1989). A very high level of polymorphism (more than 70%) was pointed out in several populations, while the mean value 65.2% is similar to that observed in boreal temperate-zone species (64.5%) (Hamrick and Godt, 1989). The average number of alleles per locus (2.0) was similar to temperate-zone species but higher than species with sexual and asexual way of reproduction, long-lived perennial plants and lower than species of widespread geographic range (2.29) (Hamrick and Godt, 1989).

Table 6
Matrix of Nei's genetic distance (above diagonal) and identity (below diagonal).

Population	A	D	F	I	M	N	K	BES	GR
A	–	0.028	0.005	0.007	0.081	0.001	0.119	0	0.009
D	0.972	–	0.026	0.036	0.050	0.007	0.139	0.016	0
F	0.995	0.975	–	0.009	0.061	0	0.073	0	0.014
I	0.993	0.964	0.991	–	0.044	0.007	0.105	0.002	0.028
M	0.922	0.951	0.941	0.957	–	0.045	0.037	0.039	0.075
N	0.999	0.993	1.000	0.993	0.956	–	0.060	0	0.011
K	0.888	0.870	0.929	0.901	0.963	0.942	–	0.069	0.152
BES	1.000	0.984	1.000	0.998	0.962	1.000	0.933	–	0.007
GR	0.991	1.000	0.986	0.973	0.928	0.989	0.859	0.993	–

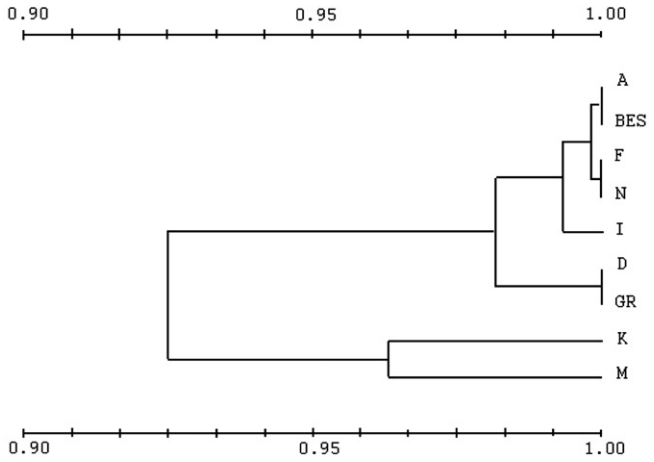

Fig. 7. Dendrogram showing allozyme differentiation between populations of *Ambrosina bassii*. Scale = Nei's (1978) unbiased genetic identity (cluster analysis using unweighted pair group method). For explanation of population codes see Table 1.

On the basis of our data, fragmentation has not led to the expected loss of genetic variation. Furthermore, there has been no apparent increase in inbreeding in fragmented populations. The maintenance of genetic variation in disjunct populations might be explained arguing that since fragmentation there has not been enough time for processes such as genetic drift and inbreeding to erode genetic variation, or for mutation and genetic drift to generate differences between populations (cf. Young et al., 1993). In Sicily, habitat fragmentation resulted from human modification of the environment: most of the hills where *Ambrosina* populations could occur have been in fact converted to cultivated lands since Roman period (about 2000 years b.p.) at least. From a genetic perspective, population size could have not been much affected by habitat fragmentation.

Moreover, the role of clonal structure of populations in maintaining a high genetic diversity has to be considered. Indeed, the present data tend to support the importance of vegetative and clonal reproduction in *Ambrosina*, as suggested by other authors (Ellstrand and Roose, 1987)

4.2. Inter-population diversity

Genetic differentiation measures (F_{st}) are rather low (Table 5) and, as a consequence, genetic flow (Nm) is significant (1.98). The occurrence of a possible genetic flow could be confirmed also by the lack of spatial genetic structure (Fig. 8). The low genetic distances between populations (Table 6, Fig. 7) points out for an overall genetic homogeneity. Indeed, it is possible to identify two main clusters: one including most of the populations sampled, and a second including "M" ("*bassii*") and "K" ("*reticulata*") populations, with no private alleles detected in "K" and "M" populations (Table 2).

On the basis of our data, fragmentation has not led to differences between populations. The similarities between populations could either be due to presently acting forces (pollen and seed dispersal?) or to the (relatively) recent more continuous distribution. In this latter hypothesis, similar cases in other long-lived perennial plants are reported (e.g. *Cytisus villosus*, Troia et al., 1997, in which the importance of the way populations become isolated on resulting genetic structure is emphasized).

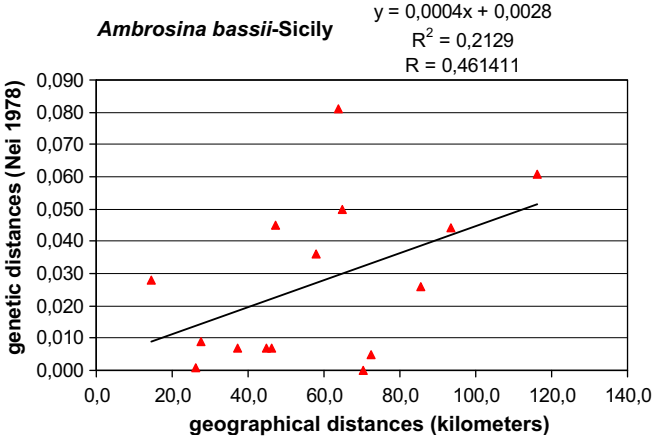

Fig. 8. Correlation between geographical distances (in km) and genetic distances between populations; a positive correlation exists, but it's a very weak, as expressed by low values of R (0.461411) and R^2 (0.2129).

4.3. Taxonomic aspects

The comparison between the morphologically distinct populations (described in the past as distinct taxa) did not show any genetic difference: the conservation of these morphological variants could suggest a certain degree of separation without a noticeable genetic differentiation (Grant, 1981; Crawford, 1990; Bancheva et al., 2006; Noel et al., 2007). Even though the different "morphotypes" co-occur in the same (or in adjacent) areas, without evident geographical or ecological barriers, the reproduction biology, although not well known (lack of pollinators? Clonal structure?), could lead towards an of-fact reproductive isolation. On the basis of the acquired data, the placement of these taxa at the varietal rank, as proposed by Giardina et al. (2007), appears appropriate.

Further studies are in progress to deepen the knowledge of pollination and reproduction biology, and to extend the study of the genetic diversity to the rest of the distribution area.

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