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Genetic differentiation of three species of *Matthiola* (Brassicaceae) in the Sicilian insular system

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Abstract. We examined the genetic variation of 12 isozyme loci in 14 populations of *Matthiola* (Brassicaceae) representing the geographic distribution of the species *M. incana*, *M. fruticulosa* ssp. *fruticulosa* and *M. tricuspidata* in the Sicilian insular system and the adjacent mainland areas to estimate the levels and distribution of genetic variation in the insular populations and to understand their population dynamics. The disparity in the distribution of polymorphism in populations of *M. incana* ssp. *incana* (low within populations but with high values of F_{ST} and G_{ST}) contrasts with the homogeneity in the inter-population distribution of the high genetic variation detected in *M. tricuspidata* and *M.* fruticulosa ssp. *fruticulosa*. While the low polymorphism found in *M. incana* ssp. *incana* is consistent with its origin through cultivation and the associated lack of gene flow, the Sicilian populations of the other two taxa probably derived from multiple founder events from nearby continental areas and, according to our estimates, have maintained high interpopulational gene flow. Unlike *M. incana*, the Sicilian populations of *M. tricuspidata* and *M.* fruticulosa ssp. *fruticulosa* could have survived the glaciations in refugia. This higher antiquity, together with the maintenance of abundant gene flow, largely explains their high values of genetic variation. In contrast, *M. incana* ssp. *pulchella* and *M. incana* ssp. *rupestris* have low indices of polymorphism and they are probably neo-endemics, as their distribution areas were severely affected by the Plio-Pleistocene glaciations.

Key words: Allozymes, *Matthiola*, Sicily, Mediterranean, continental islands, oceanic islands, diversification.

Numerous studies that have investigated the relationships among isozyme variation and biotic and abiotic traits in plant populations conclude that environmental (Loveless and Hamrick 1984; Graham 1997; Hamrick and Godt 1989, 1997), historical (Hamrick and Godt 1996) and phylogenetic variables (Gitzendanner and Soltis 2000, Membrives et al. 2001, Caujapé-Castells and Jansen 2003) are a driving influence in diversification processes and, consequently, in the levels and structuring of genetic variation. Therefore, understanding the diversification history of a group of organisms in its different regions of occurrence is of critical importance prior to drawing general conclusions from any comparative or evolutionary study.

Matthiola R. Br. (Brassicaceae) is a mainly Eurasiatic genus that consists of about 50 species of annual or perennial herbs or subshrubs (Appel and Al-Shehbaz 2003). By virtue of its widespread distribution in the Northern quarter of Africa, encompassing both continental islands (i. e. most of those in the Mediterranean basin) and oceanic ones (i. e. Macaronesia), *Matthiola* provides a good model system for understanding the contrasts between plant diversification dynamics in these two distinct types of insular enclaves.

Situated South West of the Italian Peninsula, Sicily is the biggest island in the Mediterranean, with a surface of almost 26,000 km^2. Its most important geographic feature is the massif of the Etna, whose 3,300 m high volcano dominates the landscape and is still active. According to Di Martino and Raimondo (1979) and Raimondo et al. (2001), its closeness to continental Europe (less than 3 km from mainland Italy through the Straits of Messina), its relief (more than 62% of its surface are mountains) and the noteworthy climatic and geological contrasts have been the major factors to shape and maintain a considerable floristic richness (about 2700 taxa), that comprises numerous endemic elements (ca. 426 taxa). Sicily limits with three archipelagos that belong to its territory: the Aeolian (with seven islets) to the North, the Egadian (with four islets), the islet of Pantelleria to the Southwest, and the Pelagian (with one volcanic islet and two calcareous ones) to the South.

According to the basic definitions of insularity, continental islands have been connected to the mainland in the past, while oceanic islands are often far from the nearest land mass and emerged from volcanic events in the sea bed. We must regard Sicily as a continental island on the grounds of the contacts with and separations from the continent in several past epochs (Di Martino and Raimondo 1979). By contrast, the Aeolian and the Pelagian archipelagos and the islet of Pantelleria should be considered oceanic, as these islands have never been connected to the mainland (Santo et al. 1995).

According to Greuter et al. (1986), four species of *Matthiola* are known in Sicily and its surroundings: *M. fruticulosa* (L.) Maire, *M.* incana (L.) R. Br. & Ait., *M. tricuspidata* (L.) R. Br. (Fig. 1) and *M. sinuata* (L.) R. Br. Unlike its congeners, which only have a single taxon in this archipelago, *M. incana* consists of three infra-specific subdivisions (Lojacono-Pojero 1888, Greuter et al. 1986): ssp. *incana* (L.) R. Br., which is widespread in other insular and continental Mediterranean enclaves, and the narrow endemics ssp. *pulchella* (Conti) Greuter & Burdet (confined to the islet of Pantelleria) and ssp. *rupestris* (Raf.) Nyman, distributed in the surroundings of Palermo (Sicily), and in the Aeolids, the Egadian and Ustica, respectively (Raimondo et al. 1994).

Allozyme polymorphisms are the most frequently used neutral molecular markers to study the levels and structuring of population genetic variation, and have constituted the basis for assessing diversification in a wide variety of plant families (see Hamrick and Godt 1989 for a comprehensive review). By virtue of this widespread use, allozymes provide an exhaustive population genetic database and represent a suitable method to generate population genetic information for Sicilian *Matthiola*.

This study presents genetic data based on protein electrophoresis for 14 populations of *Matthiola* in the Sicilian insular system. Because the sampling of nearby continental areas of distribution in this work is limited, we will mainly focus on the patterns of genetic variation in the sampled islands. Within this context, our specific objectives are (1) to estimate the levels and distribution of population genetic variation in the taxa represented, (2) to get insight into the possible mechanisms of maintenance of the genetic variation detected in the insular populations, and (3) to set the stage for a future comparison of the levels of variation of *Matthiola* in the continental archipelagos in the western Mediterranean vs. the oceanic islands of Macaronesia.

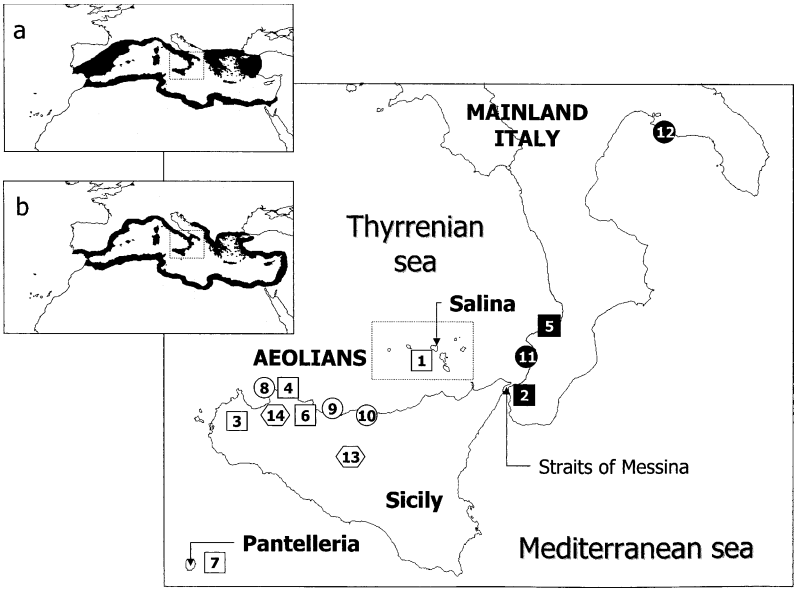

Fig. 1. Sampled localities of *Matthiola* in Sicily, Salina, Pantelleria and continental Italy. Squares: *M. incana*; circles: *M. tricuspidata*; hexagons: *M. fruticulosa* ssp. *fruticulosa*. White and black fillings signal insular and continental populations, respectively. The shadowed areas in the map of the Mediterranean correspond to the distribution of *M. fruticulosa* (**a**) and *M. tricuspidata* (**b**) according to Greuter et al. (1986) and Jalas and Suominen (1994). Numbers correspond to Table 1

Material and methods

Plant material. The species of *Matthiola* are hermaphroditic Brassicaceae with segmented fruits (siliques) that are characterised by stigma lobes often with prominent swellings or horns (Appel and Al-Shehbaz 2003). Although most Brassicaceae possess a homomorphic sporophytic self-incompatibility system (Bateman 1954, Richards 1986) that is controlled at one locus with multiple alleles (the *S*-locus), there is no available evidence regarding the reproductive system of *Matthiola*. *Matthiola fruticulosa*, *M. incana*, and *M. tricuspidata* are three diploid species that represent three different sections within the genus (*Acinotum*, *Pachynotum*, and *Aciloma*, respectively). While *M. fruticulosa* and *M.* incana are perennial sub-shrubs, *M. tricuspidata* is an annual.

Matthiola incana ssp. *incana* (common stock) has a Tyrrhenian origin, but its wide use as ornamental since century one b.c. (Saccardo 1909) caused the introduction of large amounts of material manipulated by man in this territory. In Sicily, this species (known as 'Balicu') is largely used in gardens. Because all the populations of *M. incana* ssp. *incana* sampled occur in cliffs close to inhabited areas, they are probably ornamentals that escaped cultivation. By contrast, the remaining sampled populations are from the wild.

Geographic context. Our sampling in this multi-insular system (Fig. 1) restricted to the continental island of Sicily, and the volcanic islets of Salina (a 26.8 km^2 Aeolian islet within 62 km from mainland Italy), and Pantelleria (an islet of 83 km^2 within 70 km from the Tunisian coast that constitutes the emerged part of a volcanic building). Both Pantellería and Salina, with estimated ages of ca. 220,000 years BP and 430,000 years BP, respectively (Santo et al. 1995) are much younger than Sicily.

Sampling. We sampled siliques of 14 populations of *Matthiola* (Fig. 1) representing the distribution areas of the species *M. incana*, *M.* fruticulosa and *M. tricuspidata* in Sicily (8 populations), Salina (1 population) and Pantellería (1 population), and in the mainland Italian regions of Calabria (3 populations) and Puglia (1 population). *Matthiola sinuata* was not sampled in this research owing to the extremely small size of its only known population in Sicily. We estimated that

all populations consisted of between 500 and 5,000 individuals, although those of *M. tricuspidata* often hold sizes larger than 5,000.

In all cases, siliques were collected at regular intervals along transects within each population, although when physical clumps of individuals were detected, we sampled siliques of several (or all) of the plants. For each population, the siliques collected for different individuals were put into separate envelopes that were assigned unique labels to prevent the mixture of seeds from different progenitors. The collected siliques were transported from Sicily to the Jardín Botánico Canario Viera y Clavijo, where two seeds of each silique were planted in a research greenhouse under homogeneous conditions of light, humidity and substrate.

Electrophoretic analyses. Protein extracts were made from tips of young leaves collected in the greenhouse with an extraction buffer following Shields et al. (1983). Enzyme extracts were absorbed onto Whatman n°3 paper wicks and kept at −80°C until analyzed electrophoretically. Horizontal starch gel electrophoresis was carried out with five isozyme systems, namely isocitrate dehydrogenase (IDH, EC. 1.1.1.42), malate dehydrogenase (MDH, EC. 1.1.1.37), phosphoglucose isomerase (PGI, EC. 5.3.1.9), phosphoglucose mutase (PGM, EC. 5.4.2.2) and 6-phosphogluconate dehydrogenase (6PGDH, EC. 1.1.1.44). Electrophoresis was conducted in 11% starch gels (Aldrich starch 23,402-8) in two different electrode/gel buffer systems. For 6-PGD, IDH and MDH we used morpholine-citrate 6.1 (Clayton and Tretiak 1972); systems PGM and PGI were resolved on Histidine 7.0 (Shields et al. 1983). All the staining recipes were based on Wendel and Weeden (1989) although, for some isozymes, substrate concentration and the final pHs of the staining solutions were modified to improve band resolution.

The resulting enzyme patterns were analyzed on an allelic basis. For each enzyme, gene loci and isozymes were labeled using the numerical sequence and, for each locus, alleles were labeled in alphabetical order beginning with the fastest anodally migrating alleles. The number and intensities of all bands agreed with the quaternary structure of the enzymes assayed, and the genetic bases of banding patterns were inferred from variation reported from other plants in Wendel and Weeden (1989). Intra-population, inter-population, and inter-specific verification of enzyme mobilities were determined by side-by-side comparisons of allelic variants on the same gel.

Data analyses. Calculations were made both at the population and species levels from allelic data corresponding to each locus. For *M. incana*, we considered two population subdivisions: *M. incana* sensu lato (s. l.) [i. e. all populations sampled, without considering infra-specific taxonomical subdivisions], and *M. incana* ssp. *incana* (i. e. without including the populations of ssp. *rupestris* and ssp. *pulchella*).

The number of alleles per locus (A), percentage of polymorphic loci (P), observed and expected heterozygosity (H_o and H_e) and genetic distances (Nei 1978) were calculated using BIOSYS-1 Version 1.7 (Swofford and Selander 1988) both for individual populations and for species assemblages. Nei's (1973) and Wright's (1922) population structure statistics were calculated using the computer programs Genestat-PC version 3.31 (Lewis 1993) and Popgene version 1.32 (1997), respectively. The values for Nei's (1973) statistics correspond to the unbiased estimates for population size and number (G_{ST}) and to alternative estimates to be used in cases of large amounts of divergence among the considered populations (CG_{ST}). Ewens-Watterson (EW) homozygosity tests of neutrality were run for all polymorphic loci per taxon in Popgene version 1.32 (1997). The tests constructed a 95% confidence interval for each locus included in the analyses, so that a locus can be regarded as neutral if its average EW test value falls within the corresponding interval.

An UPGMA tree was constructed with Nei's (1978) genetic distance using NTSYS-pc version 1.80 (Rohlf 1993). We used Nei's (1978) distance because simulations in Nei et al. (1983) showed this estimator to outperform other genetic distance calculation methods. The relationship among geographic location and genetic makeup for *M. incana* and *M. tricuspidata* was evaluated through Mantel tests (Mantel 1967). This is a non-parametric statistical procedure that tests the significance of the correlation between pairs of distance matrices. Mantel analyses involved matrices of geographic (D_{GEO} in km) and genetic [D_{NEI}, Nei (1978)] distances between all pairwise combinations of populations.

The different file formats needed to run the population genetic programs used to obtain the values of intra- and inter-population genetic

variation parameters were generated by the computer program Transformer-2 (Caujapé-Castells and Baccarani-Rosas 2004).

Results

Enzyme electrophoresis of the five enzymes that could be scored consistently resulted in the interpretation of 12 putative loci (the table of frequencies is available upon request to the corresponding author). Of these, only *Idh-2* and *Mdh-3* were monomorphic throughout. Of the 40 alleles scored, one (*Mdh1-b*) was exclusive to *M. incana*, six (*Pgm2-a*, *Pgm2-c*, *Idh1-a*, *Pgi2-b*, *Mdh4-c* and *6Pgd2-a*) to *M. tricuspidata*, and ten (*Pgm1-a*, *Pgm1-b*, *Pgm2-b*, *Pgm2-d*, *Pgm2-g*, *Pgm3-b*, *Pgi2-a*, *Pgi2-d*, *Pgi2-f* and *Mdh2-c*) to *M. fruticulosa*. None of these alleles was present in all populations of the corresponding species. Excluding monomorphic loci, there were nine alleles (*Pgm1-c*, *Pgm1-d*, *Pgm3-c*, *Idh1-c*, *Mdh1-a*, *Mdh2-b*, *Mdh4-a*, *6Pgd1-a*, *6Pgd2-c*) shared by all taxa. We detected four alleles shared exclusively between *M. incana* s. l. and *M. tricuspidata* (*Pgm1-e*, *Pgi2-c*, *6Pgd1-b*, and *6Pgd2-b*), four between *M. incana* s. l. and *M. fruticulosa* (*Pgm2-e*, *Pgm2-f*, *Pgm3-a*, and *Pgm3-d*) and four between *M. tricuspidata* and *M. fruticulosa* (*Idh1-b*, *Pgi2-e*, *Mdh2-a*, and *Mdh4-b*).

The highest levels of polymorphism were detected in *M. fruticulosa*, with values of maximum number of alleles per locus (A), percentage of polymorphic loci (P) and expected heterozygosity (H_e) and observed heterozygosity (H_o) of A = 2.4, P = 41.7, H_e = 0.201 and H_o = 0.177. The lowest levels of polymorphism were found in *M. incana* ssp. *incana*, with population averages of A = 1.06, P = 2.4 and H_e = 0.01 (Table 1). However, when all populations of *M. incana* ssp. *incana* were treated as a single population, the values of polymorphism (Table 1) increased sharply (A = 1.6, P = 33.3, and H_e = 0.131) by virtue of the conspicuous qualitative differences among populations. The only locus that conformed to Hardy Weinberg proportions was *6Pgd-2* (Table 3) in *M. incana* ssp. *incana*, and all polymorphic loci behaved as neutral according to Ewens-Watterson tests.

Average values of G_{ST} and F_{ST} (Table 3) were very high in *M. incana* ssp. *incana* (G_{ST} = 0.759 and F_{ST} = 0.892), and only moderate in *M. tricuspidata* (G_{ST} = 0.275 and F_{ST} = 0.250) and *M. fruticulosa* ssp. *fruticulosa* (G_{ST} = 0.193 and F_{ST} = 0.115). Estimates of gene flow through *Nm* values (Wright 1931) between pair-wise combinations of populations (data not shown) were considerably low in *M.* incana ssp. *incana* (average *Nm* = 0.05) but surpassed 1 in *M. fruticulosa* and *M.* tricuspidata (*Nm* = 3.37 and 5.46, respectively). Consistent with the values of inter-population differentiation, the highest average Nei's (1978) genetic distance (Table 2) was observed in *M. incana* ssp. *incana*, with a value of D_{NEI} = 0.113, and the lowest in *M.* tricuspidata (D_{NEI} = 0.045). The UPGMA analyses (Fig. 2) placed the three taxa in distinct clusters, and grouped ssp. *rupestris* and ssp. *pulchella* with populations of ssp. *incana*, albeit at high genetic distances in both cases. *Matthiola fruticulosa* ssp. *fruticulosa* and *M. tricuspidata* showed a closer genetic relationship (average D_{NEI} = 0.234) than either of them with *M. incana* s. l. (D_{NEI} = 0.321 and 0.340, respectively). Within *M. incana*, the genetic similarity between the populations of ssp. *rupestris* and ssp. *pulchella* as measured by Nei's genetic identity (I = 0.756) was lower than that of either of them with ssp. *incana* (I = 0.813 and I = 0.840, respectively). Mantel tests did not reveal significant correlation between the genetic and geographical distance matrices (data not shown).

Discussion

General patterns of genetic polymorphism. Population genetic studies with plants from continental islands are still rare and from few geographic areas (Affre et al. 1997, Kang and Chung 2000, Maki 2001, Sun and Wong 2001). However, reviews of the abundant allozyme literature in oceanic insular systems like the Hawaii (DeJoode and Wendel 1992), the

Table 1. Basic indicators of isozyme polymorphism for the populations of the island and mainland Italian species of *Matthiola* considered in this paper. *N*: average sample size per locus; *A*: average number of alleles per locus; *P*: percentage of polymorphic loci; H_o: observed heterozygosity; H_e: expected heterozygosity. Numerical codes correspond to Fig. 1 (map) and to the cluster. Asterisks label the mainland populations

N	Species/Locality	Code	[*N*]	[*A*]	[*P*]	[H_o]	[H_e]
M. incana ssp. *incana*							
1	Salina	IIPS	28.0	1.3 (0.1)	8.3	0.003 (0.003)	0.050 (0.041)
2	Palmi*	IIPA	20.6	1.0 (0.0)	0.0	0.000 (–)	0.000 (–)
3	Balestrate	IIBA	25.0	1.0 (0.0)	0.0	0.000 (–)	0.000 (–)
4	Monte Pellegrino	IIMP	29.0	1.0 (0.0)	0.0	0.000 (–)	0.000 (–)
5	Pizzo Calabro*	IIPC	27.0	1.0 (0.0)	0.0	0.000 (–)	0.000 (–)
M. incana ssp. *rupestris*							
6	Capo Zafferano	IRCZ	20.3	1.0 (0.0)	0.0	0.000 (–)	0.000 (–)
M. incana ssp. *pulchella*							
7	*Pantelleria*	MIPP	20.0	1.1 (0.1)	8.3	0.017 (0.017)	0.015 (0.015)
	Population average *M. incana* s. l.		24.3	1.06	2.4	0.003	0.009
	Population average ssp. *incana*		25.9	1.06	1.66	0.001	0.010
	M. incana s. l. overall		170.1	1.60	33.3	0.002	0.131
	M. incana ssp. *incana* overall		129.8	1.50	25.0	0.001	0.099
M. tricuspidata							
8	Isola delle femine	TRIF	12.0	1.3 (0.2)	8.3	0.063 (0.055)	0.052 (0.045)
9	Campofelice di Roccella	TRCR	15.7	1.3 (0.2)	16.7	0.063 (0.057)	0.074 (0.053)
10	Castel di Tusa	TRCT	26.7	1.3 (0.2)	25.0	0.093 (0.052)	0.113 (0.062)
11	San Ferdinando Calabro*	TRSF	31.3	1.7 (0.1)	33.3	0.050 (0.030)	0.144 (0.056)
12	San Isidoro*	TRSI	29.2	1.6 (0.2)	41.7	0.090 (0.044)	0.134 (0.053)
	Population average *M. tricuspidata*		23.0	1.44	25.0	0.070	0.103
	M. tricuspidata overall		114.8	2.10	41.7	0.073	0.146
M. fruticulosa ssp. *fruticulosa*							
13	Vallone Madonna degli angeli	FFMA	24.8	2.0 (0.3)	50.0	0.159 (0.068)	0.190 (0.067)
14	San Martino delle scale	FFSM	34.3	1.7 (0.3)	33.3	0.192 (0.099)	0.173 (0.083)
	Population average *M. fruticulosa*		29.5	1.85	41.6	0.175	0.181
	M. fruticulosa overall		59.2	2.40	41.7	0.177	0.201

Canaries (Francisco-Ortega at al. 2000), or the Juan Fernández (Crawford et al. 2001) provide an adequate framework to discuss (albeit by contrast) the patterns of variation detected in Sicilian *Matthiola*.

The Sicilian populations of *M. fruticulosa* hold the highest values of variation detected in this survey (average $A = 2.4$, $P = 41.7$, $H_e = 0.201$), even surpassing those of continental *M.* tricuspidata (average $A = 1.65$, $P = 37.5$, $H_e = 0.139$). Insular populations of *M. tricuspidata* also maintain moderately high levels of variation (average $A = 1.3$, $P = 16.6$, $H_e = 0.079$), though these values are roughly half those of *M. fruticulosa*. Remarkably, the amount of polymorphism in the insular populations of *M. tricuspidata* and in *M. fruticulosa* as measured by the average population diversity ($Hs = 0.077$ and 0.178, respectively [Table 3]) ranks much higher than the average reported in the Juan Fernández archipelago ($Hs = 0.042$, Crawford et al. 2001) and, in the case of *M. fruticulosa*, even higher than the average value for Canary island taxa ($Hs = 0.137$, Francisco-Ortega et al. 2000).

Because all populations of *M. incana* ssp. *incana* sampled are presumed to be associated with ornamental cultivars, it is probable that their very low genetic variation (population averages of $A = 1.06$, $P = 1.66$, $H_e = 0.01$,

Table 2. Nei's (1978) genetic distances between all pairwise combinations between the populations surveyed. Numerical codes correspond to the map in Fig 1

	[*M. incana*]							[*M. tricuspidata*]					[*M. fruticulosa*]	
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]
1. IIPS	–	0.106	0.106	0.268	0.106	0.309	0.203	0.345	0.377	0.348	0.475	0.479	0.389	0.354
2. IIPA		–	0.000	0.182	0.000	0.182	0.165	0.313	0.343	0.314	0.431	0.436	0.375	0.385
3. IIBA			–	0.182	0.000	0.182	0.165	0.313	0.343	0.314	0.431	0.436	0.375	0.385
4. IIMP				–	0.182	0.182	0.175	0.512	0.513	0.429	0.496	0.465	0.313	0.452
5. IIPC					–	0.182	0.165	0.313	0.343	0.314	0.431	0.436	0.375	0.385
6. IRCZ						–	0.280	0.512	0.513	0.429	0.496	0.465	0.460	0.568
7. MIPP							–	0.455	0.446	0.399	0.509	0.548	0.432	0.378
8. TRIF								–	0.002	0.017	0.059	0.095	0.244	0.249
9. TRCR									–	0.010	0.051	0.089	0.247	0.260
10. TRCT										–	0.046	0.073	0.218	0.246
11. TRSF											–	0.013	0.257	0.322
12. TRSI												–	0.322	0.318
13. FFMA													–	0.054
14. FFS														–

Table 1) reflects several generations of inbreeding, divergent selection, and bottlenecks rather than insularity.

However, when we consider the populations of *M. incana* ssp. *incana* as an assemblage, it is evident that they have retained a substantial amount of genetic heterogeneity (A = 1.6, P = 33.3, and H_e = 0.131) regardless of the forces that have acted to reduce variation. This effect is attributable to the fact that population IIPS possesses several polymorphic loci, and the other populations differ in the alleles that are monomorphic at loci *Pgm-1, Pgm-2*, *Pgm-3*, and *Mdh-1*. Accordingly, the high values of G_{ST} = 0.759 and F_{ST} = 0.892 in this taxon (Table 3) indicate that roughly 75% to 90% of the genetic variation resides among populations, a proportion far greater than those estimated in *M. tricuspidata* (G_{ST} = 0.275) or *M. fruticulosa* (G_{ST} = 0.193). Because of the much higher inter-population differentiation estimated in *M. incana* ssp. *incana*, the fact that the average genetic distance within this taxon (D_{NEI} = 0.113) is almost threefold the value within *M.* tricuspidata (D_{NEI} = 0.045) or *M. fruticulosa* (D_{NEI} = 0.054) is not unexpected. The F_{ST} for ssp. *incana* is also much higher than the value of F_{ST} = 0.62 reported for cultivars of ornamental *Phlox* (Levin 1977), thus indicating a prolonged action of divergent artificial selective pressures. Because all sampled populations of *M. incana* ssp. *incana* are probably reintroductions of cultivated plants into the

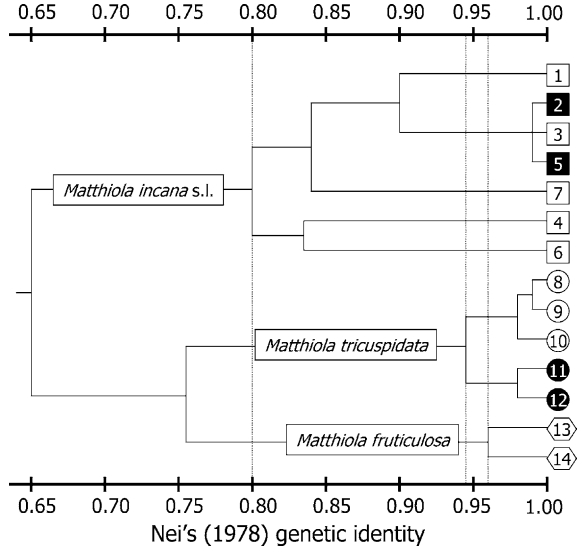

Fig. 2. UPGMA cluster of the 14 populations sampled using Nei's (1978) genetic distance. Discontinuous lines indicate the genetic distance thresholds that contain the populations of *M. fruticulosa* ssp. *fruticulosa*, *M. tricuspidata*, and *M. incana* sensu lato according to the 12 loci resolved. Symbols and numbers correspond to Fig. 1

Table 3. Chi-square tests for Hardy-Weinberg (HW) equilibrium deviations, Ewens-Watterson (EW) neutrality tests, and population structure indicators following Nei (1973) and Wright's F statistics for the 12 loci scored in the circum-Sicilian *Matthiola*. **Av**.: Average value of the EW statistic; **SE**: standard error; $\mathbf{L_{95}}$: lower limit of the 95% confidence interval for the mean value of the EW statistic; $\mathbf{U_{95}}$: Upper limit of the 95% confidence interval for the average value of the EW statistic. Only polymorphic loci are shown for each taxon

	[HW equilibrium]		[Ewens-Watterson tests]				[Nei's (1973) population structure statistics]						[Wright's F statistics]		
Taxon/locus	X^2	P	Av.	SE	L_{95}	U_{95}	Hs	Ht	Dst	CDst	G_{ST}	CG_{ST}	Fis	Fit	F_{ST}
M. incana ssp. *incana*															
PGM-1	131.0	0.000	0.844	0.027	0.508	0.992	0.000	0.400	0.400	0.511	1.000	1.000	—	1.000	1.000
PGM-2	131.0	0.000	0.841	0.027	0.505	0.992	0.000	0.400	0.400	0.511	1.000	1.000	—	1.000	1.000
PGM-3	265.1	0.000	0.719	0.035	0.384	0.977	0.298	0.529	0.225	0.390	0.425	0.518	1.000	1.000	0.779
MDH-1	257.0	0.000	0.835	0.028	0.504	0.992	0.014	0.014	0.000	0.002	0.017	0.017	1.000	1.000	0.029
PGD-2	0.000	1.000	0.832	0.028	0.505	0.992	0.007	0.007	0.000	0.000	0.000	0.002	−0.018	−0.004	0.014
Average *M. incana* ssp. *incana*							0.064	0.270	0.205	0.248	0.759	0.786	0.940	0.994	0.892
M. tricuspidata															
PGM-1	24.8	0.000	0.713	0.037	0.372	0.983	0.518	0.686	0.167	0.427	0.244	0.369	0.058	0.267	0.222
PGM-2	75.7	0.000	0.833	0.029	0.503	0.991	0.019	0.019	0.001	0.001	0.024	0.024	0.650	0.663	0.039
IDH-1	283.6	0.000	0.703	0.036	0.372	0.983	0.054	0.055	0.001	0.001	0.023	0.024	0.716	0.727	0.038
PGI-2	23.9	0.000	0.704	0.035	0.377	0.974	0.174	0.219	0.045	0.056	0.206	0.227	0.038	0.220	0.189
MDH-2	122.1	0.000	0.836	0.028	0.503	0.991	0.117	0.135	0.018	0.020	0.130	0.138	1.000	1.000	0.125
MDH-4	56.8	0.000	0.717	0.034	0.383	0.974	0.199	0.420	0.221	0.323	0.526	0.593	0.407	0.693	0.483
PGD-1	229.0	0.000	—	—	—	—	0.012	0.012	0.000	0.000	0.006	0.006	1.000	1.000	0.025
PGD-2	58.6	0.000	0.704	0.036	0.385	0.983	0.149	0.168	0.019	0.022	0.112	0.122	0.412	0.477	0.111
Average *M. tricuspidata*						0.101	0.143	0.039	0.045	0.275	0.291		0.289	0.466	0.250
M. fruticulosa															
PGM-1	51.1	0.000	0.572	0.029	0.313	0.911	0.613	0.800	0.176	0.632	0.221	0.393	0.240	0.341	0.132
PGM-2	19.0	0.041	0.487	0.025	0.264	0.842	0.140	0.152	0.010	0.012	0.064	0.070	0.184	0.218	0.042
PGM-3	34.4	0.000	0.672	0.034	0.362	0.964	0.570	0.889	0.308	1.326	0.347	0.604	−0.303	−0.019	0.218
IDH-1	0.009	0.926	0.800	0.030	0.501	0.982	0.038	0.040	0.001	0.001	0.023	0.024	−0.042	−0.020	0.020
PGI-2	8.3	0.215	0.564	0.031	0.301	0.928	0.578	0.606	0.017	0.043	0.029	0.047	−0.073	−0.047	0.023
MDH-2	165.8	0.000	0.676	0.033	0.368	0.964	0.111	0.112	0.000	0.001	0.000	0.004	0.820	0.821	0.007
MDH-4	0.090	0.764	0.809	0.029	0.502	0.982	0.090	0.100	0.009	0.009	0.084	0.089	−0.111	−0.053	0.053
Average *M. fruticulosa*							0.178	0.225	0.043	0.055	0.193	0.214	0.018	0.131	0.115

field, the continental populations (IIPA and IIPC) can be considered analogous to their Sicilian relatives (i.e. they have undergone bottlenecks, inbreeding and no migration) and, therefore, they will be of little use in the discussion of patterns of diversification in Sicily vs the continent.

Values of genetic polymorphism parameters (Table 1) in the endemic ssp. *pulchella* ($A = 1.1$, $P = 8.3$, $H_e = 0.015$) and ssp. *rupestris* ($A = 1.0$, $P = 0.0$, $H_e = 0.0$) are lower than the mean values reported by Hamrick and Godt (1989) for endemic plants ($A = 1.39$, $P = 0.26$, $H_e = 0.063$). Neither of these subspecies possesses either diagnostic or exclusive alleles with respect to ssp. *incana*, which suggests a recent origin (see below).

Despite our attempt to minimize the distances between sampled plants, the distribution of populations over wide areas might have induced a Wahlund effect (Wahlund 1928) if family structures are present in these taxa, thus explaining the observed excess homozygosity and, consequently, the deviations of the Hardy-Weinberg equilibrium detected in almost all loci (Table 3). Construing these deviations by the existence of high levels of inbreeding seems feasible only in the case of *M. incana*, given its cultivated origin. By contrast, this would be unlikely in *M. fruticulosa* and *M. tricuspidata* taking into account the adaptations of their pollen and seeds to travel long distances.

The effects of insularity. In agreement with the general expectation that insular species tend to show low values of genetic diversity as compared with close continental relatives (Frankham 1997), the levels of allozyme polymorphism in Sicilian populations of *M. tricuspidata* (TRIF, TRCR and TRCT) are lower than those in their mainland populations (Table 1). In addition, the high levels of gene flow in *M. tricuspidata* and *M. fruticulosa* as estimated through the average values of *Nm* (5.46 and 3.36, respectively) suggest that the short distance between Sicily and mainland Italy (3 Km through the Straits of Messina) must have fostered abundant genetic interchange between the two land masses in the form of both pollen and seed flow. Indeed, these taxa possess clear reproductive adaptations to facilitate dispersal: they have dehiscent fruits and their seeds are wind ballists of a tiny size that can be transported long distances by wind.

The low genetic distance (Table 2) between Sicilian populations of *M. tricuspidata* and their mainland congeners, and the clear pattern of diminishing genetic variation with increasing distance to peninsular Italy (Fig. 3) indicate that gene flow with the continent has occurred mainly within a short geographic range. This context of a high genetic interchange, together with the moderate to high values of basic indicators of polymorphism, consistently shows that one consequence of the geographic closeness to the mainland is the maintenance of considerable levels of genetic variation. Such a scenario is unlikely in oceanic insular systems because their much larger distance to the nearest land mass (and to the nearest islands) severely restricts gene flow to populations of the same island, if at all.

If proximity to the continent was in direct relationship with the amounts of polymorphism also in *M. incana*, then we should expect a reduction in the values of the indicators of genetic variation in the populations from the oceanic islands of Pantelleria and Salina by

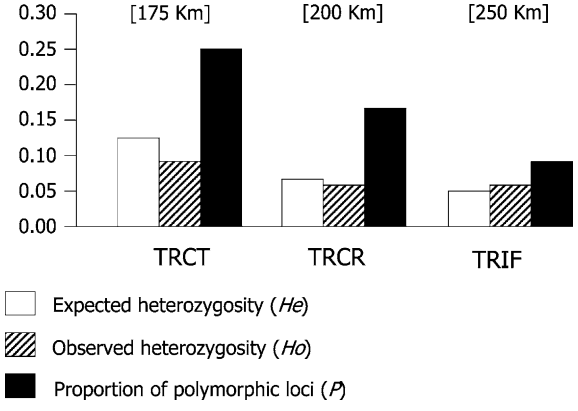

Fig. 3. Histogram showing the relationship between the basic indicators of genetic polymorphism in *M. tricuspidata* and the increasing distance to the nearest continental area

virtue of their much higher isolation (i.e. unlike Sicily, they have never been attached to the mainland). However, our data are in striking contrast with this prediction, as the populations of ssp. *pulchella* and ssp. *incana* from these islets are the most polymorphic within *M. incana* (Table 1). Although the seeds of *M. incana* are much larger in size than those of *M. tricuspidata* and *M. fruticulosa*, they possess narrow wings that permit prolonged flights, and this trait must have facilitated dispersal in ssp. *pulchella* from Pantelleria. Furthermore, the fact that this subspecies is not cultivated must have allowed it to maintain a minimum degree of genetic polymorphism. As commented above for its Sicilian and mainland relatives, the higher polymorphism of ssp. *incana* from Salina is better construed as an artefact associated with cultivation and man-mediated dispersal than as a consequence of the reproductive attributes of this species.

The fact that the endemic ssp. *pulchella* and ssp. *rupestris* are much less variable than the Sicilian *M. tricuspidata* and *M. fruticulosa* is probably accounted for by two facts. First, Pantelleria arose recently (within 220,000 years BP) through volcanic activity and has never been connected to the mainland (Agnesi and Federico 1995). Thus, the higher isolation of the population of ssp. *pulchella* must have prevented it from acquiring a high level of genetic variation through regular gene flow with the mainland.

A second important factor to explain the low genetic variation in ssp. *pulchella* and ssp. *rupestris* is the impact of glacial events in its distribution range. The vegetation in the coastal areas of Sicily and all the islets surrounding it was probably extirpated by the severe glacial cycles in the Pliocene and Pleistocene (Di Martino and Raimondo 1979). However, several mountain ranges in the North-West of the island (e. g. Madonie or Busambra) harbour monotypical genera and elements of relict flora, and are believed to have acted as refugia (Di Martino and Raimondo 1979). If we take the number of exclusive alleles detected as an indicator of relative antiquity, it is feasible that Sicilian populations of *M. fruticulosa* (with ten exclusive alleles) and *M. tricuspidata* (six exclusive alleles) are much more antique than those of ssp. *pulchella* and ssp. *rupestris* (that do not possess exclusive alleles), perhaps dating back to the time when Sicily was connected to the continent. If so, *M. fruticulosa* and *M. tricuspidata* could have survived the unfavourable periods in sheltered reducts and expanded when climatic conditions became more auspicious. In contrast, ssp. *pulchella* possibly derives from a single founder event and (like ssp. *rupestris*) is a post-glacial neoendemic that may have had much less time to generate variation. Such an interpretation coincides with that of Conte et al. (1998) for the Aeolian rare endemic *Cytisus aeolicus* Guss. (Leguminosae).

Conclusions

Unlike oceanic insular systems, where geographic isolation overrides the intrinsic biological attributes of the species to maintain genetic variation and cohesion, the disruptive actions of drift and independent mutation do not explain the population genetic polymorphism detected in the *Matthiola* taxa from the continental island of Sicily. Save for *M. incana* ssp. *incana* (whose patterns of isozyme polymorphism are attributable to its cultivated nature), a close proximity to the mainland and the seed adaptations to facilitate dispersal have acted to promote gene flow and migration in Sicilian *Matthiola*, thereby allowing the maintenance of a remarkable genetic cohesion. Although mutation could have played a differentiating role in the Sicilian populations of *M. fruticulosa* and *M. tricuspidata* by virtue of their presumable higher antiquity, the effects of this random diversification force have probably been diluted by the abundant interpopulation gene flow in these taxa.

Allozyme surveys have been conducted within the *Brassicaceae* sensu lato (Anderson and Warwick 1999) or with some of its species (e. g. Evans et al. 2000), and specimens of

Matthiola have been included in molecular phylogenetic surveys (e. g. Epping et al. 1990). However, this is (to our knowledge) the first work that studies the population genetic diversification in one of the areas of the genus distribution. The data and hypotheses presented here will be enhanced and assessed in the framework of the study of the diversification of the genus in other insular systems like the Balearic and the Canarian islands (Sánchez et al. unpubl. data) and an ongoing molecular phylogenetic analysis of the Tribe Matthioleae Schulz (Jaén-Molina et al. 2004).

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