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Orchis longicornu Poiret in Sardinia: genetic, morphological and chorological data

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Orchis longicornu Poiret in Sardegna: dati genetici, morfologici e corologici. — Nel presente lavoro sono riportati i risultati di ricerche sulla struttura genetica, sulla morfologia e sulla distribuzione di *Orchis longicornu* Poiret (1789) in Sardegna. Inoltre viene indagato il problema della presenza in quest'isola di *Orchis morio* L. (1753). Questa specie, morfologicamente simile a *O. longicornu*, è segnalata per la Sardegna nella quasi totalità dei lavori. L'analisi genetica di 27 loci enzimatici in popolazioni provenienti da località dell'isola in cui erano state citate entrambe le specie ha mostrato che nei campioni sardi i loci polimorfici sono in equilibrio di Hardy-Weinberg; tali campioni sono assai poco differenziati geneticamente tra loro: la distanza genetica media (*D*) secondo Nei è 0,01; inoltre meno del 2% della variazione genetica totale osservata è attribuibile a differenze interpopolazionali (G_{ST} = 0,015). Il flusso genico tra le popolazioni sarde risulta elevato: $Nm \approx 4$, ed è probabilmente da attribuire a trasporto passivo di semi ad opera del vento. Il confronto tra le popolazioni sarde e campioni rappresentativi di *O. morio* dell'Italia continentale mostra differenze significative nella variabilità genetica: l'eterozigosi media attesa (H_e) è rispettivamente 0,16 e 0,12. La distanza genetica tra le popolazioni della Sardegna e quelle di *O. morio* dell'Italia continentale è relativamente elevata: *D* secondo Nei = 0,18, *D* secondo Rogers = 0,22. Tra questi due gruppi di popolazioni sono state osservate differenze altamente significative nelle frequenze alleliche a vari loci (*Mdh-2*, *Sod-3*, *Pgm-1*, *Gpi-1*, *Gpi-2*). Ciascuno di tali loci dà una probabilità di identificazione corretta che varia da 0,90 a 1 (loci diagnostici). Questi risultati dimostrano l'assenza di *O. morio* nel materiale della Sardegna analizzato geneticamente; tale materiale va attribuito a un'unica specie, che verosimilmente corrisponde a *O. longicornu*. L'eterogeneità genetica, assai bassa, osservata tra le popolazioni sarde è dovuta ai loci *Est-6*, *Mdh-1*, *Dia*, *NADHdh-2*, *Pgm-1*, *Gpi-1* e *Adh*. Per alcuni di tali loci (*Mdh-1*, *Adh*, *Est-6*) essa è attribuibile a fenomeni di relativo isolamento geografico e di selezione; per altri (*Pgm-1* e *Gpi-1*) a deriva genetica. L'ipotesi che la presenza a bassa frequenza in alcune popolazioni sarde di alleli ($Gpi\text{-}1^{100}$, Dia^{107}, $NADHdh\text{-}2^{96}$) trovati in *O. morio* sia dovuta a fenomeni di paleointrogressione (sporadica immigrazione di individui di *O. morio* in Sardegna, che avrebbero diluito i loro geni nel genoma di *O. longicornu* attraverso numerose generazioni di reincrocio) non risulta sufficientemente suffragata dai dati disponibili. I dati genetici risultano in accordo con quelli morfologici. Questi ultimi sono stati ottenuti grazie all'esame di circa 1500 individui, sia vivi che essiccati, provenienti da tutta la Sardegna e dal loro confronto con esemplari d'erbario di *O. longicornu* della *patria typica* (Algeria) e con campioni di *O. morio* dell'Italia continentale. Si è preventivamente proceduto alla

tipificazione di *O. longicornu*, quindi al confronto degli esemplari sardi con quelli dell'Algeria. Gli esemplari sardi sono risultati morfologicamente simili a quelli nord-africani e sono stati attribuiti tutti a *O. longicornu*. Essi differiscono per vari caratteri (rilevati sia su materiale fresco che essiccato) da *O. morio* dell'Italia continentale. È stato messo in evidenza che alcuni di questi caratteri, usati correntemente nelle chiavi diagnostiche, si sovrappongono più o meno ampiamente nelle due specie. Ciò spiega le numerose segnalazioni di *O. morio* in Sardegna, che vanno considerate errate identificazioni di *O. longicornu*. Viene data una descrizione di *O. longicornu* della Sardegna e viene definita analiticamente la sua distribuzione nell'isola, sulla base: 1) di una revisione critica delle citazioni bibliografiche e 2) dell'esame di individui sardi sia freschi che essiccati. *O. longicornu* risulta molto ampiamente diffusa in Sardegna dove si rinviene in habitat assai diversificati, da 0 a 1500 m s.l.m.

Key words: *Orchis longicornu*, *O. morio*, Sardinia, biochemical taxonomy, multilocus electrophoresis, morphology, chorology, typification.

INTRODUCTION

The Green-Winged orchid *Orchis morio* L. (1753) and the Long-Spurred orchid *Orchis longicornu* Poiret (1789) are morphologically rather similar. Moreover, the characters currently given in the keys for their diagnosis overlap more or less widely between the two species. Accordingly, *O. morio* and *O. longicornu* have often been confused, especially in the Mediterranean area, making it impossible to assess precisely their respective ranges. *O. morio* has been recorded from Scandinavia to northern Africa (Morocco), whereas *O. longicornu* seems to be spread in the western Mediterranean region (*patria typica*: Algeria).

As to Sardinia, both *O. longicornu* and *O. morio* are currently reported (e.g. WILLIAMS, WILLIAMS & ALBOTT, 1978; SOÓ, 1980; SUNDERMANN, 1980; BAUMANN & KÜNKELE, 1982; PIGNATTI, 1982; DELFORGE & TYTECA, 1984; KURZE & KURZE, 1984; BUTTLER, 1986), as shown in fig. 1. *O. longicornu* is generally regarded as more common and widespread than *O. morio*; the presence of the latter in the island has been recently considered doubtful by some authors (RASETTI, 1980; DEL PRETE & TOSI, 1988; SCRUGLI, GRASSO & COGONI, 1988).

In the present paper, data are given on the genetic structure, morphology, and chorology of *O. longicornu* from Sardinia; furthermore, the occurrence of *O. morio* in this island is investigated, at both genetic and morphologic level. Two distinct approaches have been used: 1) the analysis of the genetic variation at 27 enzyme loci in population samples from six Sardinian locations, where both *O. longicornu* and *O. morio* were recorded, in order to test whether two distinct gene pools are present, corresponding respectively to *O. longicornu* and *O. morio*, or only a single one; 2) the morphological study of about 1500 individuals from all over Sardinia, and their comparison with specimens of *O. longicornu* from *patria typica* (Algeria) and of *O. morio* from continental Italy.

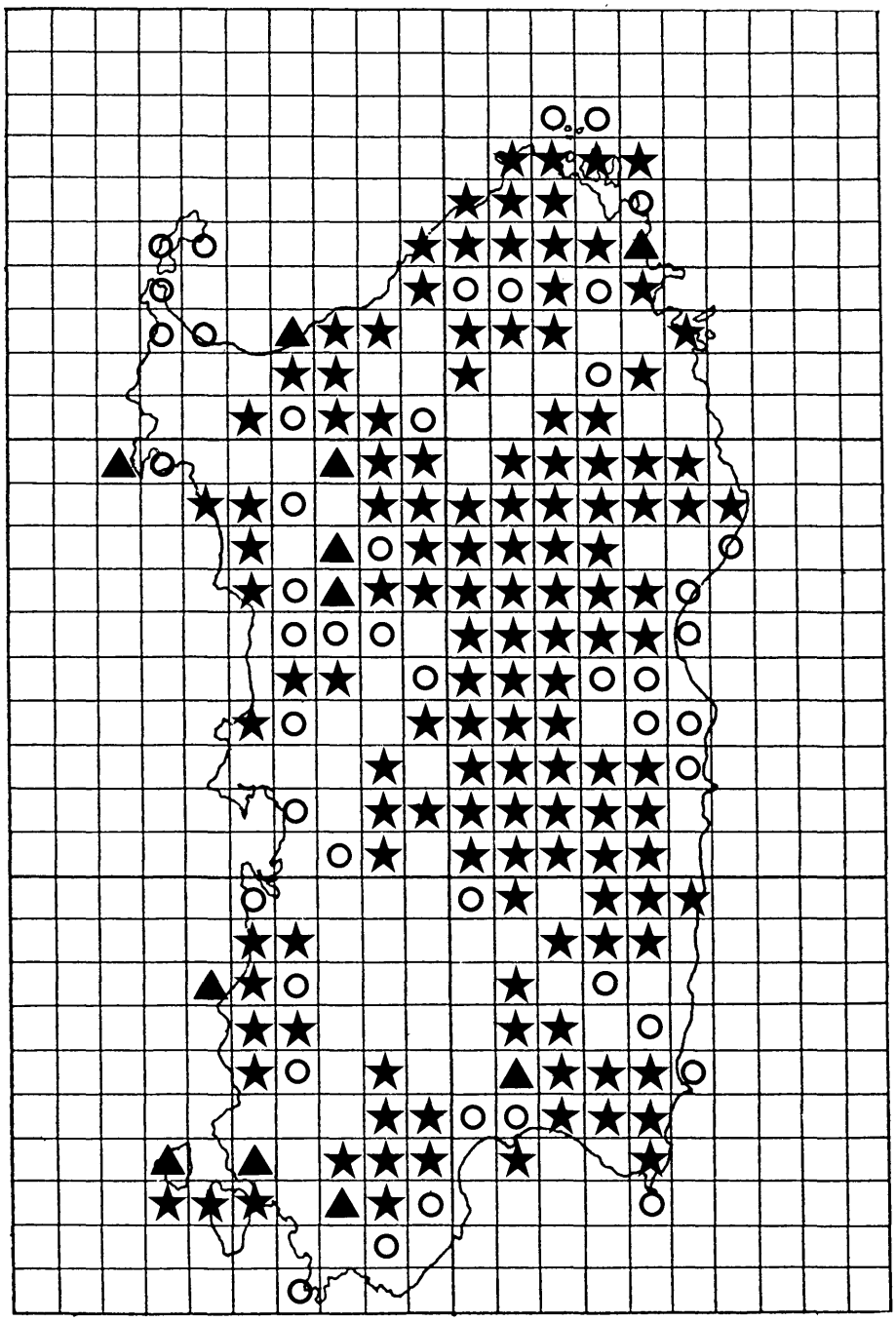

Fig. 1 - Map of Sardinia (U.T.M. grid, side 10 × 10 km) showing the locations where either *Orchis longicornu* (○), *O. morio* (▲), or both species (★) were reported from literature records.

MATERIALS AND METHODS

Multilocus electrophoresis was carried out on the following population samples from Sardinia (fig. 2). Their reference codes are given (in brackets), as well as the number of individuals tested, sampling dates and collectors.

— Monte Limbara, Vallicciola, m 1000, Tempio Pausania **(ML)**, 37 specimens, 19.5.1985 and 8.5.1988, *B. Corrias* and *S. Diana*;
— Monte Santo, m 250-300, Ardara **(MS)**, 20 specimens, 5.4.1986, *B. Corrias* and *S. Diana*;
— Chercu Arcadu, m 650, Campeda, Macomer **(CH)**, 25 specimens, 16.4.1990, *B. Corrias* and *S. Diana*;
— surroundings of Laconi, m 600, Sarcidano **(LA)**, 18 specimens, 16.4.1987, *B. Corrias* and *W. Rossi*;
— Montevecchio, m 350, Guspini **(MO)**, 42 specimens, 1.4.1984, *R. Villa*, and 12.4.1989, *B. Corrias*;
— Campanasissa, m 290, Sulcis, Siliqua **(CA)**, 20 specimens, 12.4.1988, *B. Corrias*.

Ovaries, apical stems or leaves from single individuals were used as the source of enzymes. A portion of tissue, fresh or deep frozen at −80 °C, was mechanically crushed in a grinding buffer (modified from SOLTIS, HAUFLER, DARROW & GASTONY, 1983), consisting of 0.2 M tris-HCl pH 7, 14 mM 2-mercaptoethanol, 2 mM EDTA, 1 mM dithiothreitol (DTT). The homogenates were absorbed into 3 × 5 mm chromatography paper wicks (Whatman 3MM) and inserted in 10-12% Connaught hydrolyzed starch gel trays (0.4 × 15 × 20 cm). Standard horizontal electrophoresis was performed at 6-9 V/cm for 2-5 hours at 5 °C. Gels were then cut in 2 or 3 slices, and each part stained for a specific enzyme. Details of the enzymes studied, buffer systems, and electrophoretic techniques used are given in tables 1-3.

Isozymes (i.e. enzymes with similar catalytic properties, but encoded by different gene loci were numbered in order of decreasing mobility from the most anodal (e.g. MDH-1, MDH-2, MDH-3, and MDH-4). Allozymes (i.e. enzyme variants encoded by alleles of a single gene locus) were named with numbers indicating their mobility relative to the most common allele (designated as *100*) found in a reference population of *O. morio* from Rovereto surroundings (near Garda Lake, northern Italy): alleles numbered above *100* = faster mobility of the encoded allozyme than the reference one; below *100* = slower mobility.

The following 27 enzyme loci were genetically analyzed: alcohol dehydrogenase (*Adh*), shikimic dehydrogenase (*Skdh*), malate dehydrogenase-1, -2, -3, -4 (*Mdh-1*, *Mdh-2*, *Mdh-3*, *Mdh-4*), malic enzyme (*Me*), isocitrate dehydrogenase-1, -3, -4 (*Idh-1*, *Idh-3*, *Idh-4*), 6-phosphogluconate dehydrogenase (*6Pgdh*), nicotinamide adenine dinucleotide reduced (NADH) diaphorase (*Dia*), NADH dehydrogenase-2 (*NADHdh-2*), superoxide dismutase-1, -2, -3 (*Sod-1*, *Sod-2*, *Sod-3*), glutamate-oxaloacetate transaminase-1, -2, -3 (*Got-1*, *Got-2*, *Got-3*),

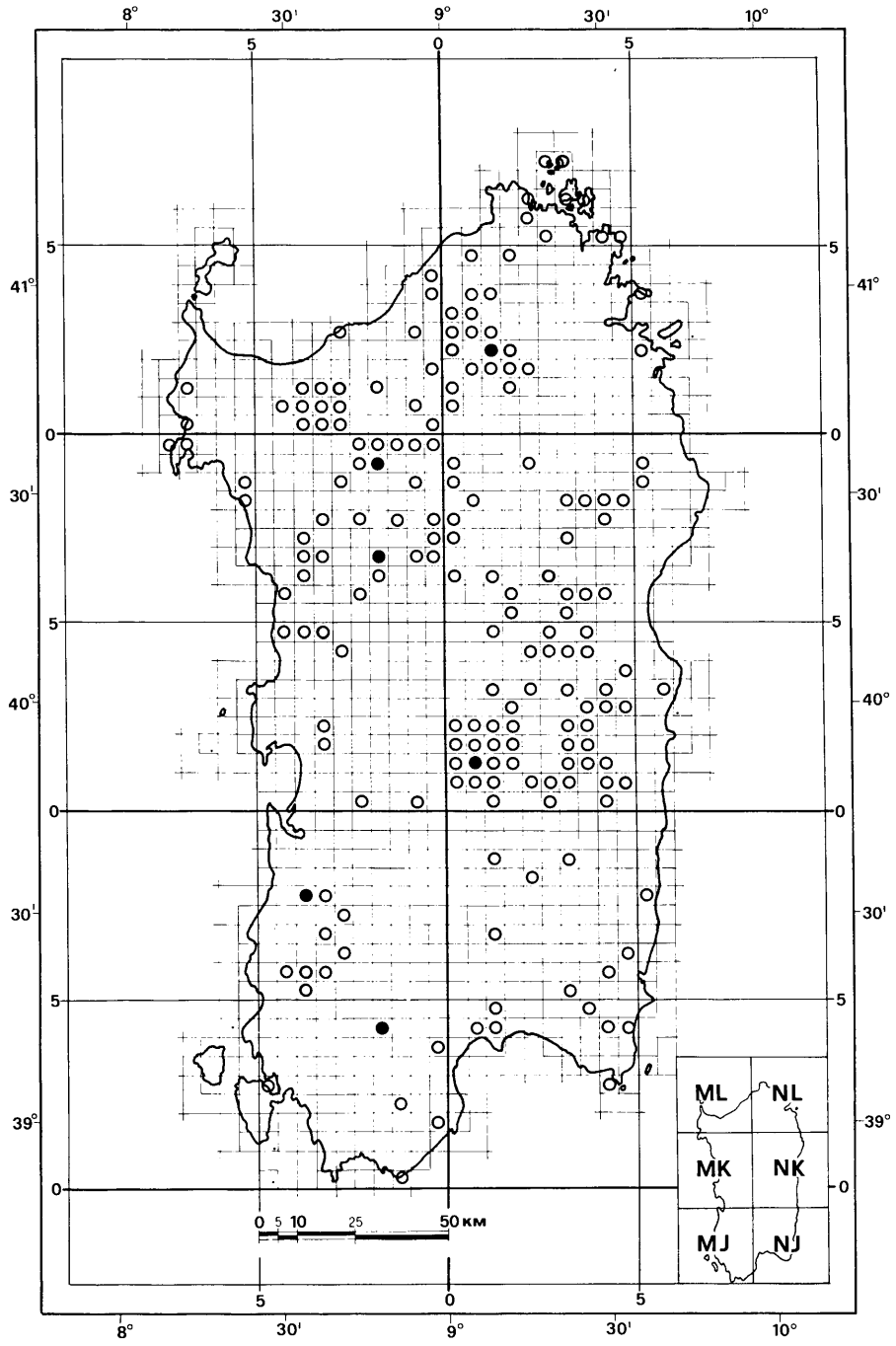

Fig. 2 - Map of Sardinia (U.T.M. grid, side 5 × 5 km), showing the collecting locations of the individuals studied morphologically (○) and of the samples analyzed both genetically and morphologically (●) in the present paper.

TABLE 1 — Enzymes essayed, encoding loci analyzed and electrophoretic migration conditions (+ = anodal migration; − = cathodal migration). For buffer system numbers see Table 2.

ENZYMES	LOCI	MIGRATION	BUFFER SYSTEMS	V/CM	TIME (H)
Alcohol dehydrogenase	*Adh*	+	6	7	3
Shikimic dehydrogenase	*Skdh*	+	7	9	5
Malate dehydrogenase	*Mdh-1*	+	3	8	3 ½
	Mdh-2	+	3	8	3 ½
	Mdh-3	+	3	8	3 ½
	Mdh-4	+	3	8	3 ½
Malic enzyme	*Me*	+	3	8	3 ½
Isocitrate dehydrogenase	*Idh-1*	+	2	7	3
	Idh-3	+	2	7	3
	Idh-4	+	2	7	3
6-Phosphogluconate dehydrogenase	*6Pgdh*	+	3	8	3 ½
NADH Diaphorase	*Dia*	+	2	7	3
NADH Dehydrogenase	*NADHdh-2*	+	4,6	7	3
Superoxide dismutase	*Sod-1*	+	5,6	7	3
	Sod-2	+	5,6	7	3
	Sod-3	+	4,5,6	7	3
Glutamate-oxaloacetate transaminase	*Got-1*	+	6	7	3
	Got-2	+	6	7	3
	Got-3	+	6	7	3
Adenylate kinase	*Adk*	+	7	9	5
Phosphoglucomutase	*Pgm-1*	+	4	7	3
	Pgm-2	+	4	7	3
Esterase (fluorescent)	*Est-1*	+	2	7	3
	Est-3	+	2	7	3 ½
Esterase (colorimetric)	*Est-6*	−	1	6	4
Glucosephosphate isomerase	*Gpi-1*	+	3	8	3
	Gpi-2	+	3	8	3

adenylate kinase (*Adk*), phosphoglucomutase-1, -2 (*Pgm-1*, *Pgm-2*), esterase-1, -3, -6 (*Est-1*, *Est-3*, *Est-6*), glucose phosphate isomerase-1, -2 (*Gpi-1*, *Gpi-2*).

The genetic variability of populations was estimated using the following parameters: proportion of polymorphic loci, P, at the 0.99 and 0.95 criteria (where 0.99 and 0.95 are the frequencies of the most common allele); mean number of alleles per locus, A; expected mean heterozygosity per locus, H_e (both unbiased and biased for small samples following NEI, 1978). Alleles with frequencies less than 0.01 were not considered for computations. Deviations

TABLE 2 — Buffer systems (analytical grade reagents per litre; pH at room temperature).

BUFFER SYSTEM	ELECTRODE	GEL
1. Lithium/borate (modified from SHAW & PRASAD, 1970)	0.03 M lithium hydroxide/0.19 M boric acid, pH 8.1 (1.20 g LiOH, 11.89 g boric acid)	electrode buffer diluted 1:10 with 0.05 M tris/0.008 M citric acid, pH 8.3 (5.59 g tris, 1.44 g monohydrate citric acid)
2. Continuous tris/citrate (SELANDER et al., 1971)	0.687 M tris/0.157 M citric acid, pH 8 (83.20 g tris, 30.00 g monohydrate citric acid)	0.023 M tris/0.005 M citric acid, pH 8 (2.77 g tris, 1.10 g monohydrate citric acid)
3. Phosphate/citrate (HARRIS, 1966)	0.15M trisodium citrate dihydrate/0.24 M sodium dihydrogen phosphate, pH 6.3 (44.11 g sodium citrate, 33.12 g NaH_2PO_4	electrode buffer diluted 1:40, adjusted to pH 6.3 with 0.2 M citric acid.
4. Tris/maleate (modified from BREWER & SING, 1970)	0.1 M tris/0.1 M maleic acid/0.01 M EDTA/0.015 M $MgCl_2$/0.125 M NaOH, pH 7.2 (12.11 g tris, 11.61 g maleic acid, 3.72 g EDTA, 3.05 g $MgCl_2$, 5.00 g NaOH)	electrode buffer diluted 1:10, pH 7.2
5. Tris/versene/borate (BREWER & SING, 1970)	0.21 M tris/0.15 M boric acid/0.006 M EDTA, pH 8 (25.40 g tris, 9.27 g boric acid, 2.20 g EDTA)	0.021 M tris/0.02 M boric acid/0.007 M EDTA, pH 8.6 (2.50 g tris, 1.24 g boric acid, 0.25 g EDTA)
6. Lithium/versene/borate (combined and modified from SHAW & PRASAD 1970, and BREWER & SING, 1970)	0.21 M tris/0.15 M boric acid/0.006 M EDTA, pH 8 (25.40 g tris, 9.27 g boric acid, 2.20 g EDTA)	electrode buffer 1 diluted 1:10 with 0.05 M tris/0.008 M citric acid, pH 8.3 (5.59 g tris, 1.44 g monohydrate citric acid)
7. Morpholine/citrate (CLAYTON & TRETIAK, 1972)	0.04 M citric acid (anhydrous), adjusted to pH 6.1 with N-(3-aminopropyl) morpholine (8.40 g citric acid, about 10 ml morpholine)	electrode buffer diluted 1:20.

from Hardy-Weinberg equilibrium were statistically analyzed with χ^2 and Fisher's exact test; LEVENE's (1949) correction was used for small samples. χ^2 and G heterogeneity tests were performed to detect significant differences in allele frequencies among samples.

The partitioning of genetic diversity within and among conspecific populations was analyzed for variable loci using NEI's (1973) estimations of H_T (total gene diversity), H_S (average gene diversity within populations), D_{ST} (average gene diversity between populations i.e. H_T - H_S), and G_{ST} (coefficient of gene differentiation, i.e. D_{ST}/H_T).

TABLE 3 — Staining recipes

ENZYME AND CODE DESIGNATION	STAINING BUFFERS	COENZYMES	ENZYMES	SUBSTRATES	ACTIVATORS INHIBITORS	VISUALIZATION METHODS
ADH EC 1.1.1.1	0.5 M KH_2PO_4/ K_2HPO_4 pH 7 10 ml + H_2O 80 ml	NAD 25 mg		95% ethanol 5 ml 2-propanol 2 ml		MTT 10 mg PMS 2 mg
SKDH EC 1.1.1.25	0.05 M tris/HCl pH 8 30 ml	NADP 7 mg		(−) shikimic acid 10 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
MDH EC 1.1.1.37	0.05 M tris/HCl pH 8 30 ml	NAD 15 mg		1 M sodium malate pH 7 5 ml		MTT 10 mg PMS 2 mg agar 0.8%
ME EC 1.1.1.40	0.05 M tris/HCl pH 8 30 ml	NADP 7 mg		1 M sodium malate pH 7 5 ml	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
IDH EC 1.1.1.42	0.05 M tris/HCl pH 8 30 ml	NADP 7 mg		DL-isocitrate 60 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
6PGDH EC 1.1.1.43	0.05 M tris/HCl pH 8 30 ml	NADP 7 mg		gluconate-6- phosphate 20 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
DIA EC 1.6.2.2	0.05 M tris/HCl pH 8 30 ml			NADH 10 mg	2,6-dichlorophenol- indophenol 3 mg	MTT 7 mg agar 0.8 mg
NADH-DH EC 1.6.99.3	0.125 M Na_2HPO_4 ·12 H_2O adjusted to pH 7 with 0.125 M NaH_2PO_4 ·H_2O 100 ml			NADH 60 mg	menadione 30 mg	MTT 20 mg

(continued)

TABLE 3 — *(continued).*

ENZYME AND CODE DESIGNATION	STAINING BUFFERS	COENZYMES	ENZYMES	SUBSTRATES	ACTIVATORS INHIBITORS	VISUALIZATION METHODS
SOD EC 1.15.1.1.						
method A	0.05 M tris/HCl pH 8 30 ml	NAD 15 mg				MTT 10 mg PMS 1 mg agar 0.8%
method B	0.05 M tris/HCl pH 8 100 ml	NADH 25 mg			menadione 25 mg	MTT 10 mg
GOT EC 2.6.1.1	0.2 M tris/HCl pH 8 100 ml			L-aspartic acid 400 mg, α-ketoglutaric acid 200 mg; adjust to pH 7.5 with 1 M tris. Pour on gel, incubate for 1/2 h, then add Fast Blue BB	Pyridoxal-5'-phosphate 20 mg	Fast Blue BB 150 mg
ADK EC 2.7.4.3	0.05 M tris/HCl pH 8 30 ml	NADP 7 mg	G6PDH (EC 1.1.1.49) 0.04 mg, HK (EC 2.7.1.1) 0.04 mg	ADP 10 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
PGM EC 2.7.5.1	0.05 M tris/HCl pH 8 30 ml	NADP 7 mg	G6PDH (EC 1.1.1.49) 0.04 mg	glucose-1-phosphate 80 mg glucose-1,6-diphosphate 2 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%
EST EC 3.1.1.1.						
(fluorescent)	0.1 M sodium/potassium phosphate pH 6.5 30 ml			4-methylumbelliferyl-acetate 5 mg in 2 ml acetone		UV
(colorimetric)	incubate in 0.5 M boric acid at 5 °C for 1 h with eserine; rinse, add 0.1 M sodium/potassium phosphate pH 6.5 100 ml			α-naphtylacetate 30 mg in 3 ml acetone	eserine 30 mg in 3 ml acetone	Fast Garnet GBC 50 mg
GPI EC 5.3.1.9	0.05 M tris/HCl pH 8 30 ml	NADP 7 mg	G6PDH (EC 1.1.1.49) 0.02 mg	fructose-6-phosphate 10 mg	$MgCl_2$ 10 mg	MTT 10 mg PMS 2 mg agar 0.8%

The values of genetic distance between populations were estimated from allele frequencies using the formulae proposed by NEI (1972) and ROGERS (1972). Unweighted pair group cluster analysis (UPGMA, SNEATH & SOKAL, 1973, NEI, 1975) was carried out using NEI's (1972) genetic distance values to show genetic relationships between populations.

The amount of gene flow between populations was estimated with the methods proposed by WRIGHT (1935, 1938, 1943, 1978) and by SLATKIN (1981, 1985) and BARTON & SLATKIN (1986), based respectively on the standardized variance of allele frequencies, F_{ST}, and on the average frequency of «private» alleles (those found in only a single population sample).

Among the morphological characters considered, the following can be mentioned: stem length; inflorescence pattern; ovary shape and length; labellum shape, colour and spotting; spur apex shape; ratio between spur length and labellum length.

The Sardinian individuals studied morphologically (about 1500) were from the locations shown in fig. 2 and listed in the paragraph «Specimina visa».

For comparison, about 60 individuals of *O. longicornu* from *patria typica* (Algeria) and about 1000 of *O. morio* from continental Italy were morphologically examined. The individuals are preserved in the following Herbaria (designation according to the International code in brackets):

— Herbarium Moris, Department of Plant Biology, University of Turin (TO);
— Herbarium Universitatis Florentinae, Botanical Museum, University of Florence (FI);
— Sardinian Herbarium, Institute of Pharmaceutical Botany, University of Sassari (SASSA);
— Herbarium of the Institute of Botany, University of Sassari (SS);
— Herbarium of the Institute and Botanical Garden, University of Cagliari (CAG);
— Herbarium of the National Museum of Natural History, Paris (P).

GENETIC DATA

Allele frequencies found at the 27 enzyme loci in the six population samples from Sardinia are given in table 4. For comparison, the average frequencies at the same loci computed on 12 population samples of *O. morio* from continental Italy are given (data from ROSSI et al., in preparation). Although the Sardinian populations genetically analyzed were from locations where both *O. longicornu* and *O. morio* had been recorded, no significant deviations were found between the observed genotype frequencies at polymorphic loci and those expected under the Hardy-Weinberg equilibrium in any of these samples (table 5). The Sardinian samples showed similar allele frequencies (see also below); on the other hand highly significant differences in allele frequencies were found at several loci (*Mdh-2*, *Sod-3*, *Pgm-1*, *Gpi-1*, *Gpi-2*) when comparing these samples

to those from continental Italy belonging to *O. morio* (fig. 3). These data indicate the absence of *O. morio* in the material examined from Sardinia: all the plants analyzed from this island apparently correspond to *O. longicornu*.

Eight enzyme loci: *Skdh*, *Idh-1*, *Idh-4*, *Sod-1*, *Sod-2*, *Sod-3*, *Got-1*, *Adk*, were found monomorphic in all the Sardinian samples. Other six loci: *Mdh-3*, *Idh-3*,

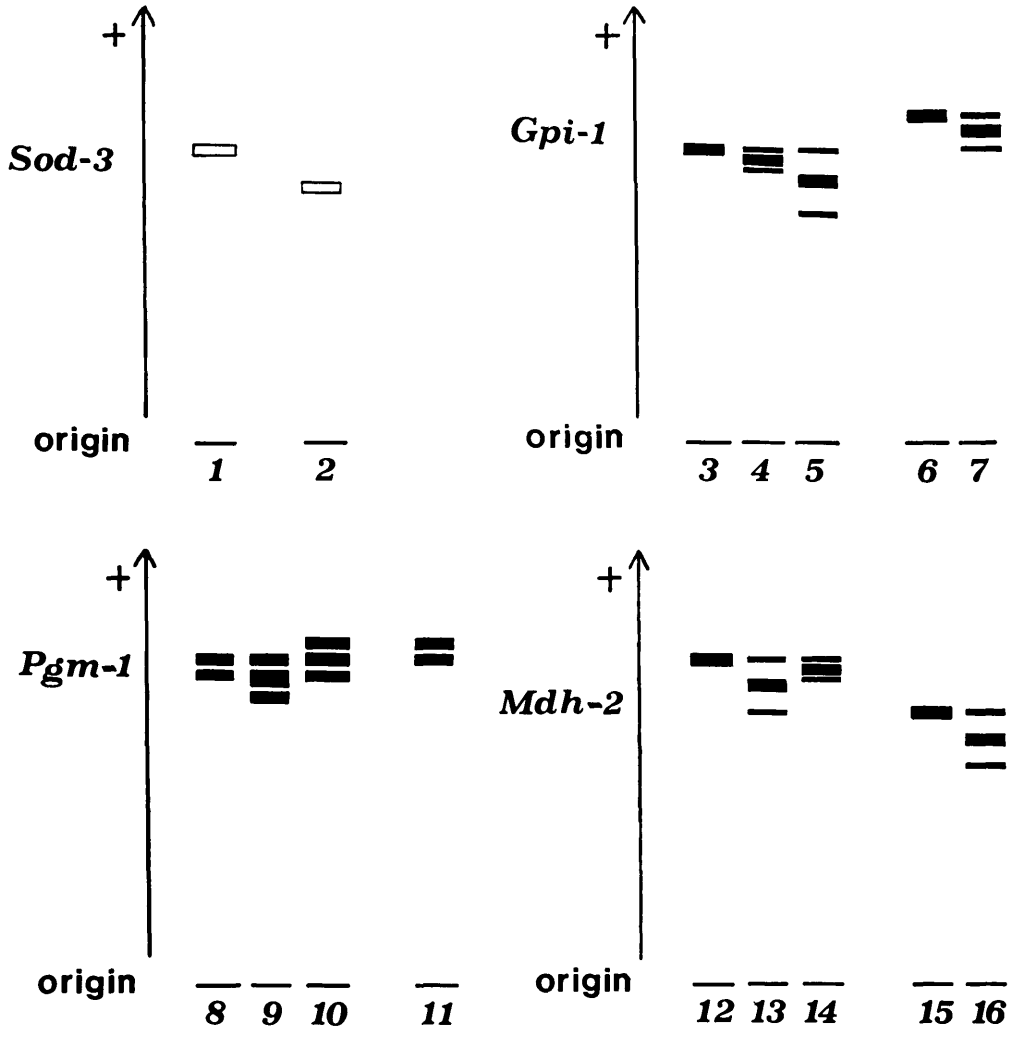

Fig. 3 - Electrophoretic pattern at the loci: *Sod-3*, *Gpi-1*, *Pgm-1* and *Mdh-2* found differentiated between *Orchis longicornu* (1, 3-5, 8-10, 12-14) and *O. morio* (2, 6, 7, 11, 15, 16). The zymograms illustrated correspond to the following genotypes. *Sod-3*: 1 = *107/107*, 2 = *100/100*; *Gpi-1*: 3 = *94/94*, 4 = *90/94*, 5 = *82/94*, 6 = *100/100*, 7 = *94/100*; *Pgm-1*: 8 = *97/97*, 9 = *93/97*, 10 = *97/100*, 11 = *100/100*; *Mdh-2*: 12 = *110/110*, 13 = *100/110*, 14 = *106/110*, 15 = *100/100*, 16 = *90/100*.

TABLE 4 — Allele frequencies at 27 enzyme loci in the six population samples from Sardinia genetically analyzed. Sample reference codes are given in the text Materials and methods. For comparison, the average frequencies computed on 12 population samples of *O. morio* from different Italian regions are given (data from ROSSI et al., in preparation)

LOCI	ALLELES	ML	MS	CH	LA	MO	CA	*O. morio*
Adh	*96*	—	0.04	—	—	—	0.04	—
	100	0.79	0.83	0.80	0.58	0.62	0.57	0.72
	106	—	—	—	—	—	—	0.02
	nul	0.21	0.13	0.20	0.42	0.38	0.39	0.26
Skdh	*96*	—	—	—	—	—	—	0.01
	100	1.00	1.00	1.00	1.00	1.00	1.00	0.99
Mdh-1	*94*	0.14	0.25	0.08	—	0.06	0.10	0.02
	100	0.86	0.75	0.92	1.00	0.94	0.90	0.96
	105	—	—	—	—	—	—	0.02
Mdh-2	*90*	—	—	—	—	—	—	0.08
	92	—	—	—	—	—	—	0.04
	100	0.27	0.06	0.28	0.32	0.35	0.30	0.87
	106	0.10	0.09	0.10	0.07	0.05	—	—
	110	0.62	0.81	0.60	0.61	0.58	0.70	0.01
	112	—	0.03	—	—	—	—	—
	114	0.02	—	0.02	—	0.02	—	—
Mdh-3	*100*	0.98	1.00	0.98	0.92	0.97	1.00	0.99
	106	0.02	—	0.02	0.08	0.03	—	0.01
Mdh-4	*92*	0.07	0.04	0.07	0.04	0.17	0.04	0.01
	96	0.04	—	—	—	0.04	—	0.01
	100	0.79	0.93	0.90	0.92	0.75	0.96	0.97
	107	0.11	0.04	0.03	0.04	0.04	—	0.01
Me	*96*	0.14	0.04	0.06	0.12	—	0.12	0.02
	100	0.86	0.96	0.94	0.88	1.00	0.88	0.98
Idh-1	*95*	—	—	—	—	—	—	0.01
	100	1.00	1.00	1.00	1.00	1.00	1.00	0.99
Idh-3	*100*	1.00	1.00	1.00	1.00	0.97	1.00	1.00
	106	—	—	—	—	0.03	—	—
Idh-4	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00
6Pgdh	*100*	0.98	1.00	0.96	1.00	1.00	0.90	0.99
	104	0.02	—	0.04	—	—	0.10	—
	108	—	—	—	—	—	—	0.01
Dia	*94*	0.02	—	—	—	—	—	0.01
	100	0.98	0.89	1.00	1.00	1.00	1.00	0.89
	102	—	0.07	—	—	—	—	—
	107	—	0.04	—	—	—	—	0.10
NADHdh-2	*96*	0.02	0.09	0.02	—	—	—	0.12
	100	0.98	0.91	0.98	1.00	1.00	1.00	0.88
Sod-1	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00

(continued)

TABLE 4 — *(continued)*.

LOCI	ALLELES	ML	MS	CH	LA	MO	CA	*O. morio*
Sod-2	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sod-3	*100*	—	—	—	—	—	—	1.00
	107	1.00	1.00	1.00	1.00	1.00	1.00	—
Got-1	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Got-2	*90*	0.04	—	—	—	0.08	—	—
	96	—	0.15	0.06	0.04	0.08	0.06	—
	100	0.96	0.85	0.94	0.96	0.84	0.94	1.00
Got-3	*96*	—	—	0.02	—	—	—	—
	100	0.79	0.81	0.83	0.96	0.92	0.72	0.99
	106	0.21	0.19	0.15	0.04	0.08	0.28	0.01
Adk	*100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pgm-1	*90*	—	—	0.02	—	—	—	—
	93	0.15	0.28	0.24	0.32	0.14	0.28	—
	97	0.75	0.63	0.71	0.43	0.69	0.67	0.01
	100	0.10	0.09	0.02	0.25	0.17	0.05	0.98
	103	—	—	—	—	—	—	0.01
Pgm-2	*92*	—	—	—	—	0.02	—	—
	94	0.02	—	—	0.05	0.02	—	0.01
	100	0.96	1.00	0.88	0.80	0.94	0.94	0.80
	105	0.02	—	0.12	0.15	0.02	0.06	0.19
Est-1	*94*	—	—	0.05	—	—	—	0.01
	100	1.00	1.00	0.95	1.00	1.00	1.00	0.99
Est-3	*96*	—	—	—	—	0.04	0.03	0.03
	100	0.98	1.00	0.97	0.95	0.89	0.95	0.65
	103	—	—	—	—	—	—	0.01
	105	—	—	0.03	0.05	0.07	0.02	0.28
	108	0.02	—	—	—	—	—	0.03
Est-6	*83*	—	—	—	—	—	0.08	—
	88	—	—	0.04	0.04	—	0.03	0.01
	93	0.77	0.69	0.92	0.75	0.50	0.50	0.10
	97	—	0.03	—	—	0.02	—	—
	100	0.16	0.06	0.04	0.14	0.29	0.21	0.44
	103	0.05	0.22	—	0.07	0.19	0.18	0.42
	105	0.02	—	—	—	—	—	0.03
Gpi-1	*82*	0.17	0.34	0.25	0.08	0.09	0.15	—
	86	—	—	0.04	0.04	—	0.02	—
	90	0.33	0.28	0.17	0.15	0.36	0.30	0.01
	94	0.48	0.38	0.54	0.73	0.54	0.53	0.05
	97	—	—	—	—	—	—	0.01
	100	0.02	—	—	—	0.01	—	0.92
	103	—	—	—	—	—	—	0.01
Gpi-2	*92*	0.17	0.21	0.04	0.14	0.25	0.17	—
	96	0.79	0.75	0.96	0.82	0.71	0.79	0.06
	100	0.04	0.04	—	0.04	0.04	0.04	0.81
	103	—	—	—	—	—	—	0.13

TABLE 5 — Chi-square (χ^2) analysis testing for deviations between genotype frequencies observed at polymorhic loci and those expected under Hardy-Weinberg equilibrium in the population samples studied. *P* = probability; m = locus found monomorhic in the population. For sample reference codes see text.

POPULATION SAMPLES		ML	MS	CH	LA	MO	CA
Loci							
Adh	χ^2	0.540	0.347	0.011	0.003	0.056	0.768
	P	*0.462*	*0.951*	*0.917*	*0.959*	*0.814*	*0.857*
Mdh-1	χ^2	0.640	1.522	0.146	m	0.133	0.181
	P	*0.424*	*0.217*	*0.703*		*0.715*	*0.671*
Mdh-2	χ^2	6.126	0.692	6.354	0.360	4.565	3.317
	P	*0.409*	*0.995*	*0.385*	*0.948*	*0.601*	*0.069*
Mdh-3	χ^2	0.000	m	0.000	0.048	0.015	m
	P	*1.000*		*1.000*	*0.827*	*0.903*	
Mdh-4	χ^2	0.844	0.040	0.181	0.048	1.078	0.000
	P	*0.991*	*0.998*	*0.981*	*0.997*	*0.982*	*1.000*
Me	χ^2	0.175	0.000	0.067	0.157	m	0.157
	P	*0.675*	*1.000*	*0.796*	*0.692*		*0.692*
Idh-3	χ^2	m	m	m	m	0.000	m
	P					*1.000*	
6Pgdh	χ^2	0.000	m	0.000	m	m	0.181
	P	*1.000*		*1.000*			*0.671*
Dia	χ^2	0.000	0.130	m	m	m	m
	P	*1.000*	*0.988*				
NADHdh-2	χ^2	0.000	0.111	0.000	m	m	m
	P	*1.000*	*0.739*	*1.000*			
Got-2	χ^2	0.000	3.126	0.073	0.000	0.312	0.000
	P	*1.000*	*0.077*	*0.787*	*1.000*	*0.958*	*1.000*
Got-3	χ^2	1.087	1.286	0.756	0.000	0.043	1.026
	P	*0.297*	*0.257*	*0.860*	*1.000*	*0.835*	*0.311*
Pgm-1	χ^2	2.457	2.316	1.702	7.386	2.126	1.170
	P	*0.483*	*0.509*	*0.945*	*0.061*	*0.547*	*0.760*
Pgm-2	χ^2	0.023	m	2.886	0.450	0.061	0.000
	P	*0.999*		*0.089*	*0.930*	*1.000*	*1.000*
Est-1	χ^2	m	m	0.000	m	m	m
	P			*1.000*			
Est-3	χ^2	0.000	m	0.000	0.000	0.331	0.027
	P	*1.000*		*1.000*	*1.000*	*0.954*	*0.999*
Est-6	χ^2	6.084	1.614	0.048	8.468	7.049	7.575
	P	*0.414*	*0.952*	*0.997*	*0.206*	*0.316*	*0.670*
Gpi-1	χ^2	6.152	6.519	5.788	1.474	3.485	6.310
	P	*0.406*	*0.089*	*0.447*	*0.961*	*0.746*	*0.389*
Gpi-2	χ^2	2.863	1.244	0.000	0.392	0.420	0.643
	P	*0.413*	*0.743*	*1.000*	*0.942*	*0.936*	*0.886*

6Pgdh, *Dia*, *NADHdh-2*, *Est-1*, showed a low level of polymorphism (frequency of the most common allele >0.90, expected mean heterozygosity ≤ 0.05). A higher genetic variation was detected at the remaining loci, and especially at *Adh*, *Mdh-2*, *Pgm-1*, *Est-6*, *Gpi-1* (expected mean heterozygosity > 0.40).

The overall genetic variability of Sardinian samples, estimated by the proportion of polymorphic loci (P_{99}, P_{95}), mean number of alleles per locus (A) and expected mean heterozygosity per locus (H_e), is summarized in table 6, together with the average values found in the 12 Italian populations of *O. morio*. The *O. longicornu* samples from Sardinia show similar values of genetic variability at all the considered parameters: P_{99} ranges from 0.48 to 0.63 (average 0.54), P_{95} from 0.37 to 0.48 (average 0.42), A from 1.8 to 2.0 (average 1.9), and H_e from 0.13 to 0.17 (average 0.16). Lower values were observed in *O. morio* from Italy ($P_{99} = 0.48$, $P_{95} = 0.35$, $A = 1.7$, $H_e = 0.12$). These values of genetic variability fall well wilhin the range observed in outcrossing plants, while being significantly higher than that found in selfing plants (HAMRICK, LINHART & MITTON, 1979; GOTTLIEB, 1981; BULLINI, ARDUINO & CIANCHI, 1989). These findings therefore demonstrate the lack of selfing in these orchids, also shown for *O. morio* by NILSSON (1984).

The analysis of genetic diversity (table 7) shows that less than 2% of the overall genetic variation observed in the Sardinian samples is attributable to differences between populations ($G_{ST} = 0.015$). The low degree of differentiation between populations from Sardinia is confirmed by the low values of

TABLE 6 — Parameters of genetic variation (P_{99} and P_{95} = proportion of polymorphic loci following the 0.99 and the 0.95 criterion respectively; A = average number of alleles per locus; H_e = expected mean heterozygosity) in the six population samples of *Orchis longicornu* from Sardinia. For sample reference codes see text. The average values calculated for the same parameters on twelve population samples of *O. morio* from Italy are given for comparison (data from ROSSI et al., in preparation).

POPULATION SAMPLES	P_{99}	P_{95}	A	H_e
O. longicornu				
ML	0.63	0.37	2.0	0.16
MS	0.48	0.44	1.9	0.16
CH	0.63	0.44	2.0	0.13
LA	0.48	0.37	1.8	0.15
MO	0.52	0.44	2.0	0.17
CA	0.52	0.48	1.9	0.17
average	*0.54*	*0.42*	*1.9*	*0.16*
O. morio				
average	*0.48*	*0.35*	*1.7*	*0.12*

TABLE 7 — Partitioning of genetic diversity within and among populations (NEI, 1973) in *Orchis longicornu*. H_T = total gene diversity; H_S = average gene diversity within populations; D_{ST} = average gene diversity among populations; G_{ST} = coefficient of gene differentiation (= D_{ST}/H_T). Only variable loci were considered.

LOCI	H_T	H_S	D_{ST}	G_{ST}
Adh	0.429	0.419	0.010	0.023
Mdh-1	0.188	0.180	0.008	0.042
Mdh-2	0.499	0.494	0.005	0.010
Mdh-3	0.051	0.050	0.001	0.020
Mdh-4	0.231	0.230	0.001	0.004
Me	0.150	0.150	0.000	0.000
Idh-3	0.009	0.009	0.000	0.000
6Pgdh	0.049	0.048	0.001	0.020
Dia	0.041	0.040	0.001	0.024
NADHdh-2	0.043	0.042	0.001	0.023
Got-2	0.158	0.158	0.000	0.000
Got-3	0.271	0.270	0.001	0.004
Pgm-1	0.512	0.504	0.008	0.016
Pgm-2	0.148	0.146	0.002	0.013
Est-1	0.017	0.017	0.000	0.000
Est-3	0.080	0.080	0.000	0.000
Est-6	0.490	0.465	0.025	0.051
Gpi-1	0.613	0.602	0.011	0.018
Gpi-2	0.333	0.329	0.004	0.012
average	*0.227*	*0.223*	*0.004*	*0.015*

genetic distance (table 8). Nei's D ranges from 0.005 to 0.020 (average D = 0.011), Rogers' D from 0.041 to 0.086 (average D = 0.059). When comparing the samples from Sardinia with those of *O. morio* from continental Italy, the average values of genetic distance are much higher: Nei's D = 0.177, Rogers' D = 0.223 (Rogers' index weights more than Nei's one slight differences in allele frequencies, as implied by the different algorithms used). The genetic relationships shown by the Sardinian *O. longicornu* samples between each other and with Italian *O. morio* are summarized in the UPGMA cluster given in fig. 4.

The slight heterogeneity found between Sardinian samples is mainly due to the loci *Est-6*, *Mdh-1*, *Dia*, *NADHdh-2*, *Pgm-1*, *Gpi-1* and *Adh*. For some of these loci the pattern of allele distribution may be related to geographical variation, suggesting a role of isolation and selection with local differences, possibly adaptive: e.g. *Mdh-1*^{94} reaches higher allele frequencies in the northern populations; *Adh* shows a clinical variation (although not significant with the analyzed sample size), with increase of the frequency of the *nul* allele (coding for an enzyme electrophoretically not active) along a north-south gradient;

TABLE 8 — Matrices of values of genetic distance (below the diagonal) and genetic identity (= similarity, above the diagonal) between six population samples of *Orchis longicornu* from Sardinia, calculated respectively with the formulas by NEI, 1972 (above) and ROGERS, 1972 (below). For sample reference codes see text.

	ML	MS	CH	LA	MO	CA
ML	—	0.992	0.995	0.989	0.992	0.993
MS	0.008	—	0.990	0.980	0.985	0.989
CH	0.005	0.011	—	0.990	0.986	0.989
LA	0.012	0.020	0.010	—	0.990	0.990
MO	0.008	0.015	0.014	0.010	—	0.993
CA	0.007	0.011	0.011	0.011	0.007	—
ML	—	0.945	0.959	0.941	0.948	0.956
MS	0.055	—	0.936	0.914	0.924	0.935
CH	0.041	0.064	—	0.942	0.933	0.945
LA	0.059	0.086	0.058	—	0.941	0.945
MO	0.052	0.076	0.067	0.059	—	0.950
CA	0.044	0.065	0.055	0.055	0.050	—

NADHdh-2^{96} was detected only in northern Sardinia, whereas *Est-6* shows the lowest frequency of *93*, together with the highest frequencies of *100* and *103*, in southern Sardinia. At other loci, such as *Pgm-1* and *Gpi-1*, the interpopulation pattern of variation involves differences of allele frequencies in single populations, such as Ardara and Laconi, possibly reflecting phenomena of

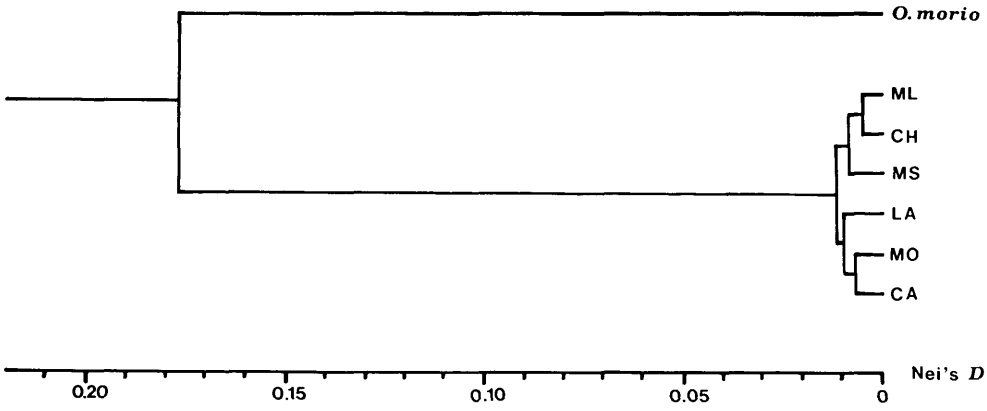

Fig. 4 - UPGMA cluster (based on Nei's *D* matrix in Table 8), showing the genetic relationships of *Orchis longicornu* Sardinian samples among each other and with Italian *O. morio* (average value over 12 population samples from continental Italy, data from Rossi *et al.*, in preparation). Nei's *D* in linear scale following standard representation (see Nei, 1975).

genetic drift. These two populations show the highest average values of *D* and the lowest values of P_{99} and *A* among the Sardinian samples tested. A hypothesis for the finding at a low frequency of some alleles: *Gpi-1*^{100} (at ML and MO), *Dia*^{107} (at MS) and *NADHdh-2*^{96} (at ML, MS and CH), that are present in *O. morio*, involves palaeointrogressive phenomena through sporadic immigrants (e.g. seed transported by wind) of the latter species. *O. morio* alleles would have been diluted in the Sardinian *O. longicornu* genome via many generations of backcross. This hypothesis, that would imply a free gene exchange between *O. morio* and *O. longicornu*, is not sufficiently supported by the available data.

The rate of gene flow between the populations from Sardinia, as expressed by the product *Nm* (where *N* is the effective population size and *m* the proportion of migrant individuals) was estimated from allele frequencies using the «private alleles» method of SLATKIN (1981) and BARTON & SLATKIN (1986), and the «standardized variance of allele frequencies» method of WRIGHT (1935). The two approaches yielded similar results: $Nm = 4.18$ with the first method, and 3.72 with the second one. These values indicate high migration rates, possibly related to seed dispersal by wind.

MORPHOLOGIC DATA

The material from Sardinia was compared with herbarium specimens of *O. longicornu* from Algeria and with live and herbarium specimens of *O. morio* from continental Italy. For a better understanding of the possible differences existing at the morphological level between *O. longicornu* from Sardinia and from its *patria typica* (Algeria), we consider it useful to typify this species.

TYPIFICATION OF O. LONGICORNU — Abbot Poiret, during his trip in «Barbarie» (Algeria) in the years 1785-86, collected a new orchid in slightly humid and shadowy sites near the sea («sur les bords de la mer, dans les lieux un peu humides et ombragés»); he considered it similar to *O. morio* («elle approche beaucoup de l'*Orchis morio*») and named it *O. longicornu*. The Poiret Herbarium is now kept in Paris at the Muséum National d'Histoire Naturelle (P), where specimens were studied by one of us (B.C.) in 1985. Exsiccata by Poiret relative to *O. longicornu* from northern Africa are comprised in two collections:

1) Herbier de Lamarck (P-LA): there is only one sheet with one fragment of stem and two fixed specimens, accompanied by three labels: a) «*orchis longicornu/ poiret/ dic. n 24*» (*dic.* = Dictionnaire), b) «*côte de Barbarie/ rac. 2*

bulbes arrondis», and c) «*côte de Barbarie*». The first label is handwritten by Poiret, whereas the other two by Lamarck. These specimens are to be considered syntipi.

2) Herb. E. Cosson (P): there is one sheet with 2 single leaves and 5 fixed specimens, each with its own label (fig. 5). The specimen fixed on the lower right side (which comes from the Herb. Poiret in Herb. Moquin-Tandon) has a handwritten label by Poiret: «*orchis bulbis indivisis, nectarii/ labio trifido subcrenulato/ cornu longo obtuso ascendente,/ petalis conniventibus/ orchis longicornu/ (nobis)*», with «Poiret» added by someone else. The description is identical with that published by POIRET (1789).

DESIGNATIO TYPI — The above described specimen — the plant on the lower right side (fig. 5) — kept in P, is here designated as *lectotypus* of *Orchis longicornu* Poiret, Voy. Barbarie 2: 247 (1789).

About 50 other exsiccata from Algeria have been morphologically studied.

SPECIMINA VISA — Environ d'Alger, s.d., *s. coll.* (P) — Oran, s.d., *Muby* (FI). — Collines environ de Philipperville, 28.2.18??, *Meyer* (FI). — Algeria, 5.1837, *Roussel* (P). — Alger collines, 3.1839, *Bové* (P). — Alger... fin d'avril 1840, *Baran* (P). — Broussales avoisant la Montagne des Lions, près d'Oran, 28-2-1852, *Balansa* (FI). — Collines des environs de Bone, fevrier-mars 1864, *Dukerley* (FI). — Environ d'Alger, 3.1864, *Durando* (FI). — Plateau du Hamma, près d'Alger, 14.2.1877, *Meyer*, (FI). — Dans les bois a Saoula près d'Alger, 4.1884, *Allard* (FI). — Bois des Nador à Urbanie près Médeat, Algerie, 22-4-1892, *Gabert* (FI).

The morphological comparison of *O. longicornu* individuals from *patria typica* with those from Sardinia did not show significant differences; the populations from the two regions should therefore be considered as conspecific. On the other hand, none of the specimens collected in Sardinia corresponds morphologically (or genetically) to *O. morio*, including the so-called *O. morio* var. *picta*. The main characters differentiating *O. longicornu* from *O. morio*, as shown by the population samples examined, are listed in table 9.

One of the best differential characters, especially in fixed individuals, is the shape of the inflorescence appearing lax in *O. longicornu* and dense in *O. morio*, owing to the different bending of the ovaries which determines the spatial location of the flowers. In fresh specimens good but not fully diagnostic characters are the contrasting colours between the lateral lobes and the central part of the labellum, the spot number and arrangement and, to a lesser extent, the labellum bending downwards. On the other hand, characters usually given in keys, such as the ratio between spur and labellum length, or the relative length of median and lateral lobes, show a lower diagnostic value, owing to their intra- and interpopulation variability that causes a considerable overlap between the two species.

A description of *O. longicornu* from Sardinia, based on the study of about 1500 individuals (either alive or dried) from all over the island, is given below.

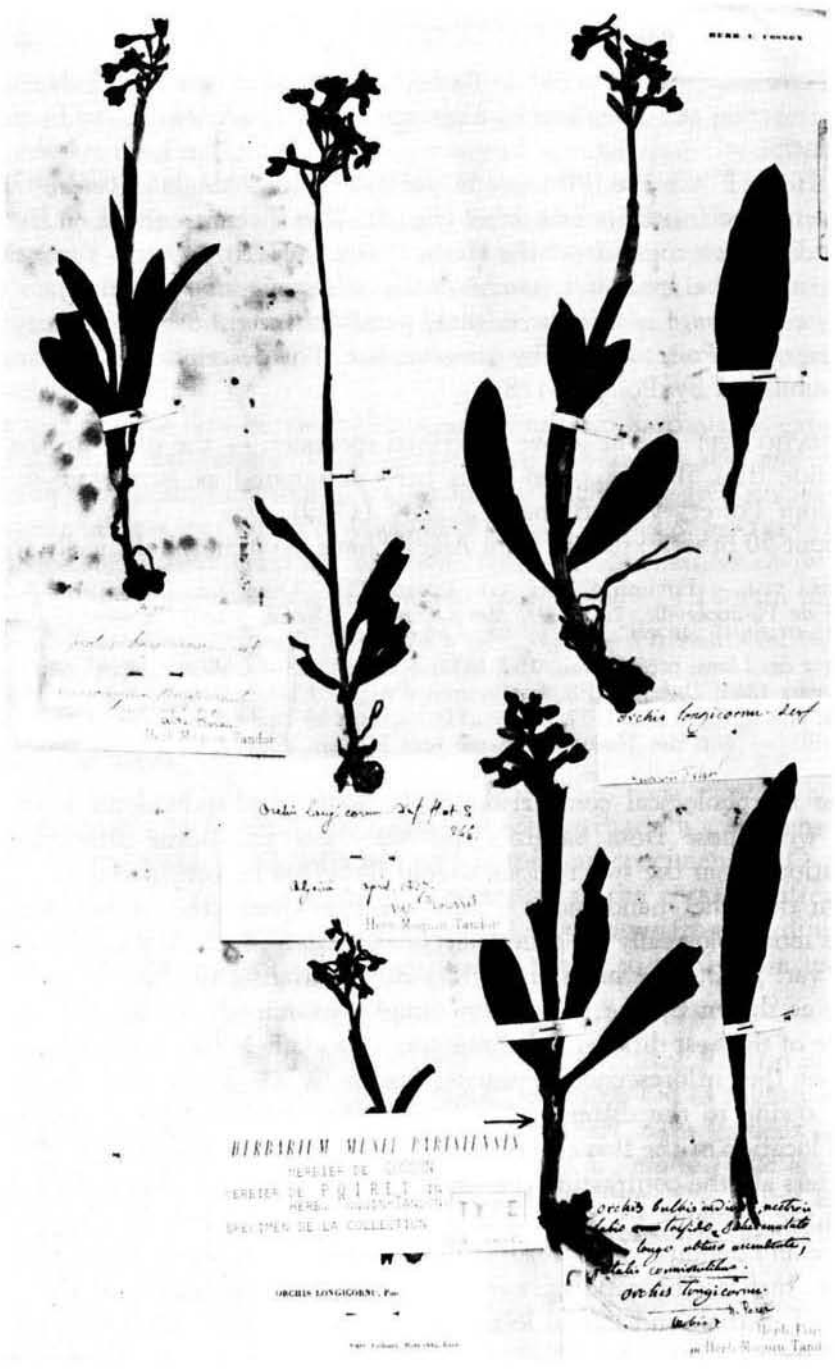

Fig. 5 - The lectotype of *Orchis longicornu* Poiret (1789): specimen in the lower right side. Sheet from Herbier E. Cosson, kept in P (Photo by G.G. Aymonin).

TABLE 9 — Main morphological characters differentiating *Orchis longicornu* from *O. morio*, with a different degree of diagnostic value.

	O. longicornu	*O. morio*
INFLORESCENCE	lax, with flowers spreading from the stem	dense, with flowers close to the stem
OVARY	recurved in a wide arc	generally recurved only in the distal portion
LABELLUM	central area paler, sharply contrasting with lateral lobes, scarcerly spotted, spots generally aligned in two rows	central area weakly contrasting with lateral lobes, densely spotted, spots disposed on several rows
	lateral lobes generally reflexed downwards	lateral lobes more or less folded downwards
	central lobe entire, often shorter than or subequal to lateral ones	central lobe generally indented, almost always as long as or longer than lateral ones
SPUR	from slightly longer than labellum to two and a half times longer	from as long as labellum to almost twice as long.

DESCRIPTION — Herbaceous perennial plant, 15-25 (35) cm high. Tubers: two, ovoid or subglobose, one sessile and one shortly stipitate. Stem slightly angled, surrounded at the base by whitish sheaths. Basal leaves forming a rosette, from elliptic to oblong-lanceolate, sub-obtused or mucronate, the superior ones sheathing and shorter. Inflorescence relatively short, oblong, lax, with a variable number of flowers (5-17), with colour varying from white-pinkish to deep purple. Bracts oblong-lanceolate, shorter than the ovary. Ovary twisted and broadly curved, holding the flower away from stem. Sepals often green veined, joining with the two petals, which are shorter and linear, to form an obtuse hood. Labellum wider than long, with a sligthly irregular margin, clearly 3-lobed; median lobe entire, often shorter than or subequal to the lateral ones, white or pinkish with scarce purple spots more or less regularly arranged in two rows. Lateral lobes rhomboid-rounded, usually much darker than the rest of the flower, reflexed downwards. Spur from slightly longer than the labellum to two and a half times longer, cylindrical, often dilated and flat at the apex, curved upwards or horizontal.

CHROMOSOME NUMBER — $2n = 36$, observed on specimens from Orotelli, Nuoro (SCRUGLI, DE MARTIS & MULAS, 1976).

DISTRIBUTION AND ECOLOGY OF ORCHIS LONGICORNU IN SARDINIA

In order to assess the range of *O. longicornu* in Sardinia, we have taken into account: 1) literature records, referred to either *O. longicornu* or *O. morio* (fig. 1); 2) the morphological study of about 1500 herbarium specimens; their collecting locations are shown in fig. 2. However, it has been possible to find the exsiccata referring to literature records of *O. morio*, (see below in the section «Specimina visa») in only a few cases.

Literature records sub Orchis longicornu:

MORIS, 1827: frequent in the woods. — GENNARI, 1866: in chestnut woods of Aritzo. — GENNARI, 1870: Caprera at Mt Tejalone. — MACCHIATI, 1881: Sassari, Baddimanna, Capuccini, Bunnari, Scala di Cioga, Mt Fiocca, Padro, Osilo, Porto Torres, Nurra. — BARBEY, 1884: Muravera, Mt S. Angelo, S. Antioco, Punta Trebina, S. Lussurgiu, Cagliari, Macomer, Aritzo, Ingurtosu, Flumini Maggiore, Gairo. — BINNA, 1886: Sassari: Cudinei, Rizzeddu, Caniga. — MARCIALIS, 1889: Cagliari outskirts. — GENNARI, 1890: Cagliari: Promontorio S. Elia, Mt Urpino, Bonaria. — COLOMO, 1893: Mt Sette Fratelli. — VACCARI, 1894: Maddalena Island at Cala Chiesa. — MARTELLI, 1896: Sassari, Osilo at Bonaria, Tonara, Donori, Castiadas, Domus de Maria. — NICOTRA, 1897: on Sassari Plateau. — CAVARA, 1901: Donori, Muravera. — BICKNELL, 1904: Macomer, Tresnuraghes, Iglesias. — FALQUI, 1905: Capoterra Mountains. — VACCARI, 1908: Maddalena, Caprera, S. Stefano, Spargi, S. Maria, Razzoli, Budelli. — TERRACCIANO, 1910: Sassari: Baddimanna, S. Simplicio, Cudinei, Serra Niedda, Serra Secca, Filigheddu, Rizzeddu, Spartivento, S. Martino, Osilo at Serra de Coloras, Mt Gonare, Limbara, Oristano at Santu Barzolu, Capoterra. — NANNETTI, 1914: Osilo at Mt Erosu. — MOLA, 1916: Bosa. — MOLA, 1919: Bosa: Mt Mannu, P. Trivides, Piano Ma-Marri, Mt Sa Pittada, Tinniri, Balviu Mannu, Mt Furru, Mt Alvo. — NEGODI, 1927: Asinara. — NEGODI, 1931: Mt Rasu, Limbara. — COSSU, 1949: Sarcidano, Isili, Montresta, Macomer. — MARTINOLI, 1950: Capo S. Elia. — MARTINOLI, 1953: Is Arenas. — MARTINOLI & PIRODDI, 1956: Mt Tuttavista. — ROVINETTI, 1957: Mt Ortobene. — DESOLE, 1959: Nurra of Porto Torres, Scala Erre. — ARRIGONI, 1964: Pixinamanna Forest. — VALSECCHI & CORRIAS, 1967: Mt Rasu. — KLINGER, 1974: Ulassai. — CAMARDA, 1976: Orgosolo at Costa sos Venales. — MILIA & MOSSA, 1976: S. Antioco Island. — SCRUGLI & GRASSO, 1979: Sarcidano. — CAMARDA & BALLERO, 1981: Capo Carbonara. — ARU *et al.*, 1982: Rio S'Acqua Callenti basin. — ANGIOLINO & CHIAPPINI, 1983: Mt Linas. KURZE & KURZE, 1984: widespread in Sardinia, in 145/270 observed squares. — CAMARDA,1984a: Mt Albo. — CAMARDA, 1984b: Mt Gonare. — BALLERO & BOCCHIERI, 1987: Capo Teulada at Brallisteris. — ATZEI & DRASCICH CAMPAZZI, 1988: Bunnari. — BALLERO, 1988: Capo Ferrato. — SCRUGLI, GRASSO & COGONI, 1988: Sarcidano. — BALLERO, CHIAPPINI & SECHI, 1988: Mt Santo. — BOCCHIERI, 1988: Asinara Island. — BOCCHIERI, MULAS & AVENA, 1988: Capo Mannu at Mesalonga. — MULAS, 1990: Mt Arci.

Literature records sub Orchis morio:

MORIS, 1827: in woodlands. — MACCHIATI, 1880: Sassari at Baddimanna. — BARBEY, 1884: Sardinia. — BINNA, 1886: S. Anatolia. — PRUNAS TOLA, 1886: Sardinia. — COLOMO, 1893: Sette Fratelli Forest. — FALQUI, 1905: Capoterra Mountains, S. Elia, Sinnai, S. Leone. — TERRACCIANO, 1910: volcanic hills around Bunnari basin, Mt S. Antonio on the slope in front of Mt Santo roadtender's house (cant.), Mt Santo near Ardara. — SANNA, 1935: widespread in the woods and in the grasslands of Logudoro. — DESOLE, 1956: Marina di Lioneddu. — CHIAPPINI, 1962: mouth of the Rio Silis. — CHIAPPINI, 1964: Mt Unturzu at Sa Fraigada. — DE MARCO &

MOSSA, 1973: S. Pietro Island. — VERI & BRUNO, 1974: Mt Limbara at Vallicciola. — SCRUGLI, DE MARTIS & MULAS, 1976: Escalaplano. — KURZE & KURZE, 1984: widespread in Sardinia, in 134/270 observed squares.

Specimina visa

In silvis frequens, martio maio (Sardinia), s.d., *Moris* (TO). — In herbidis, martio, s.d., *Moris* (TO). — Muravera, Monte S. Angelo, S. Antioco, P.ta Sa Trebina, S. Lussurgiu, Cuglieri, Sulcis in herbidis frequens, martio aprili, s.d., *Moris* (TO, SASSA). — Cuglieri, in pascuis maritimis, s.d., *Moris* (TO, sub *O. longicornu* var.?). — Sardinia, s.d., *Moris* (TO, sub *O. longicornu* var. *picta*). — In humentibus, aprili, s.d., *Moris* (TO, sub *O. longicornu* aut *O. morio* var?). — In..., junio, s.d., *Moris* (TO, sub *O. morio*? aut *O. picta*?). — In collinis, aprile, s.d., *Moris* (TO, sub *O. morio*). — Isole intermedie, aprile maggio, 1837, *Lisa* (TO, sub *O. longicornu* aut *O. picta*?). — Laconi, 2.4.1864, *s. coll.*, (TO). — Cagliari, *s.d.*, *Minio* (FI). — Is Pinnettas, in montibus inter Cagliari et San Vito (Sarrabus), 17.4.1872, *s. coll.* (FI). — Giovannibono, prope Monte Narba, Sarrabus, in nemoris submontanis, 22.4.1872, *Sommier* (FI). — Inter Ussasai et Seui, 29.4.1872, *Sommier* (FI). — A Laconi, Sardegna, 17.5.1879, *Biondi* (FI). — A Laconi nel Sarcidano, a 630 m, 17.5.1879, *Biondi* (FI). — Cappuccini, Sassari, Sardegna, 15.3.1881, *Macchiati* (FI). — Insulae Sardiniae, reg. bor. In aridis incultis prope Castel Doria, 3.4.1884, *Forsyth-Major* (FI). — In Monte S. Giovanni, prope Iglesias, 3.3.1885, *Forsyth-Major* (FI). — In umbrosis ad Capo Carbonara, 7.3.1885, *Forsyth-Major* (FI). — Capo Spartivento prope Domus de Maria, 22.4.1889, *Martelli* (FI). — Raunolta, nei dintorni di Aritzo, 19.5.1890, *Fiori* (FI). — Monti di Baunaei a Genna Ilisci, 1894, *Lovisato* (FI). — Ghiagaro, Cagliari, presso Cannias, 1894, *Lovisato* (FI). — Bruncu Aranbiu, Sarrabus, 1894, *Lovisato* (FI). — Sponde del Rio Mandari, 1894, *Lovisato* (FI). — Donori in herbosis sylvaticis, 5.4.1894, *Martelli* (FI). — In herbosis et umbrosis Monte Marganai, 8.4.1894, *Martelli* (FI). — Praterie presso Castiadas, 10.4.1894, *Martelli* (FI). — Prope Castiadas, 10.4.1894, *Martelli* (FI). — Tonara in herbosis Montis Su Dini, 23.5.1894, *Martelli* (FI). — Bonanno, presso Osilo, 13.5.1895, *Martelli* (FI). — Sette Fratelli..., 18.4.1896, *Martelli* (FI). — Belvì, 27.5.1896, *Martelli* (FI). — Sassari, s.d., *Nicotra* (FI). — Orune, 5.1.1899, *Martelli* (FI). — Presso Campuomo, 5.1900, *Cavara* (FI). — Tempio Pausania, lungo il Rio Mularza, 21.4.1903, *Gestro* (FI). — Insula Maddalena, haud rara in locis apricis, solo siliceo, alt. 1-200 m, *Vaccari* (FI). — Sull'orlo dei boschi a San Leone, Capoterra, s.d., *Falqui* (SASSA, sub *O. morio*). — Bosco del Colonnello Prunas, 19.3.1907, *Terracciano* (SASSA). — Sassari a Baddimanna, 21.3.1907, *Terracciano* (SASSA). — Sassari a San Simplicio, 25.3.1907, *Terracciano* (SASSA). — Oristano, Monte Santu Barzolu, 29.3.1907, *Terracciano* (SASSA, sub *O. morio*). — Sassari a Serra Secca, 7.4.1907, *Terracciano* (SASSA). — Sassari a Serra Niedda, 7.4.1907, *Terracciano* (SASSA). — Filigheddu, 25.4.1907, *Terracciano* (SASSA). — A Rizzeddu, valletta presso il bosco Prunas, 25.4.1907, *Terracciano* (SASSA). — Colline attorno ai Bagni di San Martino, 29.4.1907, *Terracciano* (SASSA). — Osilo, Serra de Coloras, 4.5.1907, *Terracciano* (SASSA). — Colli vulcanici attorno al bacino di Bunnari, 14.5.1907, *Terracciano* (SASSA, sub *O. morio*). — Spartivento, 14.5.1907, *Terracciano* (SASSA). — Rizzeddu, 22.3.1908, *Terracciano* (SASSA). — Rizzeddu, bosco del Colonnello Prunas, 22.3.1908, *Terracciano* (SASSA). — Monte S. Antonio, pendici verso la cantoniera di Monte Santo a levante, 12.4.1908, *Terracciano* (SASSA, sub *O. morio*). — Monte Santo, versante nord, 13.4.1908, *Terracciano* (SASSA, sub *O. morio*). — Monte Gonare, 21.4.1908, *Terracciano* (SASSA). — Limbara, 26.5.1909, *Poeverlain* (SASSA). — Da Berchidda per «su capu de sa terra» e Funtana de Inzas a S. Alvera, 16.4.1910, *Terracciano* (SASSA). — Sassari a Baddimanna, 16.3.1912, *Fiori* (FI). — Pula, salendo al M. Santo, granito, m 100 ca., 23.3.1912, *Fiori* (FI). — Macomer, Tanca Manna, 21.5.1913, *Terracciano* (SASSA). — Santu Lussurgiu, altipiano ad eriche e geniste dalla fontana Tumbarenaiu sino alla fine di Cianchevelu du, 25.5.1913, *Terracciano* (SASSA, sub *O. morio*). — Santu Lussurgiu, altipiano ad eriche e geniste dalla fontana Tumbarenaiu sino alla fine di Cianchevelu du, 25.5.1913, *Terracciano* (SASSA). — Santu Lussurgiu, da rocce Sos Banditos alle acque di Zalelavru, 25.5.1913, *Terracciano* (SASSA). — Montagna di Bolotana da Frida alla Serra e Funtana su Niberu, 30.5.1913, *Terracciano* (SASSA). — Gruppo Sette Fratelli, Sarrabus, San

Gregorio, 8.3.1925, *Negri* (FI). — Circondario di Aritzo, 1935, *Porru* (FI). — Pixinamanna (Pula), 16.3.1959, *Arrigoni* (FI). — Sarcidano. Esterzili, M.te Santa Vittoria, m 730-1212, 22.5.1963, *Bavazzano & Ricceri* (FI). — Sarcidano. Gadoni, foresta di Gadoni, rocce di Su Taono, 22.5.1963, *Bavazzano & Ricceri* (FI). — Sarcidano. Laconi, nuraghe Genna Corte, m 494, 22.5.1963, *Bavazzano & Ricceri* (FI). — Sarcidano. Laconi, rocce di Funtanamela, m 650 ca., 22.5.1963, *Bavazzano & Ricceri* (FI). — Catena del Marghine. Anela, S'Isfundadu, 13.5.1965, *Corrias* (SS). — Aggius. Piana di Aggius, 4.4.1966, *Valsecchi* (SS). — Villacidro. Foresta Montimannu. Sotto la Punta Planu Tidilis e Punta Planu Cardu, 11.5.1967, *Arrigoni & Ricceri* (FI). — Seui. Foresta demaniale di M. Arbu, da Middai ad Anulù, 12.5.1967, *Arrigoni & Ricceri* (FI). — Seui. Foresta demaniale di M. Arbu, rio sotto la caserma forestale, 12.5.1967, *Arrigoni & Ricceri* (FI). — Bono. Monte Rasu, zona cacuminale, 16.5.1967, *Corrias* (SS). — Laconi. Bosco di Funtanamela, m 710, 17.5.1967, *Alias* (FI). — Laconi. Funtanamela, 19.5.1967, *Alias* (FI). — Laconi. Foresta in loc. Duccau, m 800-850 ca., 23.5.1967, *Alias* (FI). — Laconi. Gariga da Palaxiu Monsignore a Cubeddu, 19.4.1968, *Alias* (FI). — Laconi. Lecceta in loc. Su Lau, 19.4.1968, *Alias* (FI). — Lula. Monte Albo, Punta Catirina, m 1127, 10.4.1969, *Cumpostu* (SS). — Belvì. Noccioleti presso Bau Desulo, 3.5.1969, *Arrigoni & Ricceri* (FI). — Desulo. Pascoli sopra Arcu Tascusì, m 1250-1300, 3.5.1969, *Arrigoni & Ricceri* (FI). — Orgosolo. Pascoli di Pratobello, 3.5.1969, *Arrigoni & Ricceri* (FI). — Badde Salighes. Pendici nord di Sa Sierra, 4.5.1969, *Arrigoni & Ricceri* (FI). — Tempio Pausania. M.te Limbara, torrente fra La Madonnina e Vallicciola a nord della strada, 13.5.1969, *Arrigoni & Ricceri* (FI). — Tempio Pausania. Monte Limbara, rocce e prati cacuminali nei dintorni di P.ta Balestrieri, 13.5.1969, *Arrigoni & Ricceri* (FI). — Seui. Foresta di Montarbu, 4.4.1970, *Alias* (SS). — Olbia. Rocce e garighe di capo Figari, Golfo Aranci, 9.4.1970, *Arrigoni & Ricceri* (FI). — Tempio Pausania. Versante nord di M.te Limbara in loc. Giacumeddu, m 1150, 4.6.1970, *Arrigoni & Raffaelli* (FI). — Tempio Pausania. Monte Limbara vers. NO di Punta Giugantinu sopra Vallicciola, 8.5.1971, *Arrigoni & Nardi* (FI). — Piana di Aggius, 10.5.1971, *Dolcher* (SS). — Gonnosfanadiga. Pascoli culminali di Monte Linas, 20.5.1971, *Angiolino* (FI). — Guspini, 4.4.1972, *Floris* (SS). — Sadali, 5.4.1972, *Ligios* (SS). — Ossi. Su Littu, 1.5.1972, *Corrias* (SS). — Nuoro, 6.4.1973, *Sotgiu* (SS). — Buddusò, 10.4.1973, *Chessa & Satta* (SS). — Tempio. La Fumosa, 16.4.1973, *Azara* (SS). — Tempio Pausania. Monte Limbara, pendici verso il lago, 19.4.1973, *Corrias & Diana* (SS). — Trinità d'Agultu, inizio strada bianca per Li Cossi, 2.3.1974, *Castiglia* (SS). — In agro di Escalaplano (Nuoro), 24.3.1974, *Scrugli, Mulas & De Martis* (CAG, sub *O. morio* ssp. *morio*). — Villagrande Strisaili. Pascoli sul versante ovest di M.te Pipinari, da m 1230-1350 ca., 26.4.1974, *Arrigoni, Mori & Nardi* (FI). — Jerzu. Rocce e pendici calcaree di M.te Lunburau, 2.5.1974, *Arrigoni, Mori & Nardi* (FI). — Jerzu. Rupi e pendici calcaree dei Tacchi, presso la chiesa di S. Antonio, 3.5.1974, *Arrigoni, Mori & Nardi* (FI). — Prov. Nuoro. Tacco di Osini, prati su calcare, 3.5.1974, *Arrigoni, Mori & Nardi* (FI). — Sassari, Molafà, 15.3.1975, *Becca* (SS). — Sassari, Bunnari, 28.3.1975, *Cossu* (SS). — Villagrande Strisaili, 1.4.1975, *Melis* (SS). — Limbara, 14.4.1975, *Villa* (SS). — Talana, 4.5.1975, *Corrias* (SS). — Bultei. Monti di Bultei, Orrosile, ericeto, 9.5.1975, *Corrias* (SS). — Cuglieri. Cima del Monte Ferru, poco sopra La Madonnina, suolo basaltico, m 1000, 30.5.1975, *Arrigoni, Nardi & Di Tommaso* (FI). — Siligo. Pendici nord di Monte Santo, 20.3.1976, *Corrias* (SS). — Alghero. Porto Conte, pineta Mugoni, 21.3.1976, *Corrias* (SS). — Alghero. Monte Timidone, versante NW, 4.4.1976, *Corrias* (SS). — Castelsardo. S.S. 134, al km 21, 25.4.1976, *Corrias* (SS). — Codrongianus. S.P. 597 Saccargia-Oschiri, km 8-13, 25.4.1976, *Corrias* (SS). — Piana di Aggius. Grandi Sassi, 28.4.1976, *Corrias* (SS). — Giara di Gesturi, 1.5.1976, *Corrias* (SS). — Campeda al km 155, 4.5.1976, *Corrias* (SS). — Campeda, scalo ferroviario, 4.5.1976, *Corrias* (SS). — Badde Salighes, 19.5.1976, *Corrias* (SS). — Orani. Monte Gonare, Janna Otheu, 23.5.1976, *Corrias* (SS). — Orani. Monte Gonare, prati boscati salendo alla sella, 23.5.1976, *Corrias* (SS). — Tempio Pausania. M. Limbara, Conca de li Banditi, 23.5.1976, *Camarda* (SS). — Trinità d'Agultu. Li Cossi, 27.2.1977, *Corrias* (SS). — Usini, 27.2.1977, *Cossu* (SS). — Lula. Monte Albo, 5.4.1977, *Camarda* (SS). — Lago Bidighinzu, 19.4.1977, *Corrias* (SS). — Cagliari. S. Basilio, 20.4.1977, *Corrias* (SS). — Orgosolo. Foresta di Montes, loc. Cavarcone verso S'ena longa, 22.4.1977, *Corrias & Diana* (SS). — Orgosolo. Foresta di Montes, pascolo Cavarcone,

22.4.1977, *Corrias* (SS). — Foresta Burgos, 27.4.1977, *Corrias* (SS). — Orgosolo. Supramonte all'inghiottitoio, 30.4.1977, *Dolcher* (SS). — Oliena. Lungo la strada per Su Cologone, 2.2.1978, *Corrias* (SS). — Tonara. Complesso Vatyo, loc. Sa Tanca de S'Appisorgia, 9.3.1978, *Alias* (SS). — Ozieri, 8.3.1978, *Pastorino* (SS). — Oliena. Lungo la strada provinciale per Su Cologone, 19.3.1978, *Alias* (SS). — Magomadas, 2.4.1978, *Angioi* (SS). — Mara. Bono Ighinu, Tuva e Mari, 2.4.1978, *Bertelli* (SS). — Ozieri. Strada Oschiri-Sassari n° 579, bivio per Martis, loc. Burghidu, 9.4.1978, *Sanna* (SS). — Oliena. Strada Oliena-Orgosolo, ponte Neosula, 22.4.1978, *Alias* (SS). — Domusnovas. Strada per la miniera di sa Duchessa, dopo la grotta di San Giovanni, scisti, 26.4.1978, *Corrias* (SS). — Strada Trinità d'Agultu-Aggius, km 4,2, 29.4.1978, *Corrias* (SS). — Tempio Pausania. Monte Limbara, salendo a Vallicciola, 29.4.1978, *Corrias* (SS). — Seui. Strada Ussasai-Villanova, prima di Genna e Medau, bivio per Montarbu, 1.5.1978, *Mucedda* (SS). — Ussasai. Rio Taquisara, prima della stazione, scisti, 1.5.1978, *Mucedda* (SS). — Montevecchio, sughereta, 12.5.1978, *Corrias* & *Diana* (SS). — Olzai. Strada bianca Olzai-Mamoiada, 17.5.1978, *Diana*, *Valsecchi* & *Villa* (SS). — Sassari. Palmadula, 4.3.1979, *Campus* (SS). — Sassari. Lago di Baratz, sponda NO, 18.3.1979, *Manca* (SS). — Villagrande Strisaili. Lago Flumendosa alto, 23.3.1979, *Diana* (SS). — Bitti. Santuario dell'Annunziata, 25.3.1979, *Camarda* (SS). — Buddusò. Padru, pendici di M. Nieddu, loc. Piattu, 25.3.1979, *Sechi* (SS). — Siligo. Strada per Ardara, loc. Mesomundu, 25.3.1979, *Sanna* (SS). — Oschiri. S.S. 199 Oschiri-Monti, km 22-23, 28.3.1979, *Manca* (SS). — Perfugas. Erula, 28.3.1979, *Sechi* (SS). — Bortigiadas. S.S. 127, km 59, prati ai bordi della strada, 2.4.1979, *Corrias* (SS). — Ardara. Strada Sassari-Oschiri n° 579 presso il bivio per Ardara, 7.4.1979, *Sanna* (SS). — Ozieri. Strada Oschiri-Sassari presso il bivio per Chilivani, Cant. Figos, 7.4.1979, *Sanna* (SS). — Tula. Sponde del lago Coghinas, 7.4.1979, *Sanna* (SS). — Cossoine, 10.4.1979, *Pinna* (SS). — Onanì. Riu Mannu. loc. Ganghé, 13.4.1979, *Camarda* (SS). — Lodé. Monte Albo, C. Guzzurra presso fontana Talisi, 14.4.1979, *Camarda* (SS). — Siniscola. Monte Albo, Fontana Sa Mela, 13.4.1979, *Camarda* (SS). — Talana, lungo la strada Talana-Lorotzai, al km 10, loc. Sa Mola, granito, 14.4.1979, *Corrias* & *Diana* (SS). — Orgosolo. Pratobello, loc. Sa 'E Zarminu, 16.4.1979, *Corrias* & *Diana* (SS). — Orotelli. Lungo la S.S. Trasversale Sarda, al km 67, loc. Tanca Noa, 16.4.1979, *Corrias* & *Diana* (SS). — Villagrande Strisaili. Sponda sinistra del Lago alto Flumendosa, loc. Corgiale, m 800, 16.4.1979, *Corrias* & *Diana* (SS). — Fonni. 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Doneiddu, 30.4.1979, *Corrias* & *Diana* (SS). — Laconi. S. Sofia, loc. Casidragiu, 30.4.1979, *Corrias* & *Diana* (SS). — Laconi. Strada per Funtana Raminosa, loc. S'Incrastu e Sa Fudda, 30.4.1979, *Corrias* & *Diana* (SS). — Sadali. Strada prov. n° 8 Gadoni-Seulo, km 34,5, loc Sa Xenobida, 30.4.1979, *Corrias* & *Diana* (SS). — Seulo. Strada prov. n° 8 Gadoni-Seulo, km 18, loc. Nardoa, 30.4.1979, *Corrias* & *Diana* (SS). — Meana Sardo. Presso Ponte Malifattu, S.S. 128, km 75, 1.5.1979, *Corrias* & *Diana* (SS). — Chiaramonti. M. Sassu, m 640 s.l.m., 5.5.1979, *Sanna* (SS). — Gonnosfanadiga, 5.5.1979, *Zurru* (SS). — Orgosolo. Foresta di Montes, da Dispensa de Truncuriai a Sas Venas sul Flumineddu, 17.5.1979, *Corrias* & *Diana* (SS). — Aritzo. Sarcidano. Prati nei pressi della Cant. Ortuabis, 23.5.1979, *Corrias*, *Diana* & *Camarda* (SS). — Gadoni. Sarcidano. Bauzzoni, sotto S'Angionadore. Macchia ad eriche ed *Arbutus*. Calcare, 23.5.1979, *Corrias*, *Diana* & *Camarda* (SS). — Laconi. Sarcidano. Is Casteddos, 23.5.1979, *Corrias*, *Diana* & *Camarda* (SS). — Laconi. Sarcidano. Strada per la miniera Su Sarmentu, Croce di Gandola, 23.5.1979, *Corrias*, *Diana* & *Camarda* (SS). — Bono. Foresta di Burgos, prima del bivio per il passo, loc. Brotzu, m 825, 8.3.1980, *Corrias* & *Diana* (SS). — Alghero, presso la

strada per Valverde, 15.3.1980, *Roggero* (SS). — Lula. Monte Albo, macchie sotto Punta Turuddò, 1.4.1980, *Camarda* (SS). — San Vito. Strada statale 125 Cagliari-Muravera, km 46, prima del ponte di Monte Acuto, 4.4.1980, *Corrias & Diana* (SS). — Sinnai. S.S. 125, loc. Sette Fratelli, 4.4.1980, *Corrias & Diana* (SS). — Castiadas, 5.4.1980, *Corrias & Diana* (SS). — Strada Villasimius-Castiadas, prima del bivio di S. Pietro, 5.4.1980, *Corrias & Diana* (SS). — Ierzu, 6.4.1980, *Corrias & Diana* (SS). — Ulassai, presso il paese, 6.4.1980, *Corrias & Diana* (SS). — Villaputzu. Strada Muravera-Tertenia, km 82, presso Cant. S. Barbara, 6.4.1980, *Corrias & Diana* (SS). — Strada Oliena-Orgosolo, km 4, loc. Golonica, 7.4.1980, *Corrias & Diana* (SS). — Strada Orientale Sarda, km 163, tra Baunei e Cant. Giustizieri, 7.4.1980, *Corrias & Diana* (SS). — Villanova Monteleone. Strada Alghero-Bosa, loc. Mandra Sa Perda, 13.4.1980, *Valsecchi* (SS). — Gonnosfanadiga. Monte Linas, Punta Camedda, m 950-1000, 20.4.1980, *Angiolino* (SS). — Santu Lussurgiu. Loc. Badde Urbara, pratelli e zone di macchia a *Genista* sp., 21.4.1980, *Camarda* (SS). — Laconi. S.S. 128, km 71,1, loc. Senerizzi, 1.5.1980, *Corrias, Diana & Camarda* (SS). — Orgosolo. Supramonte, loc. Ischina e Malaviu, 3.5.1980, *Camarda* (SS). — Tempio Pausania. M. Limbara, salendo a Vallicciola, loc. Multaragna, m 950-990, 17.6.1980, *Corrias & Valsecchi* (SS). — S. Teresa di Gallura. Conca Verde, Stazzo Cunchedda, 10.3.1981, *Serra* (SS). — Arzachena, Porto Cervo, residui di macchia lungo la circonvallazione, 15.3.1981, *Corrias & Diana* (SS). — Bortigiadas. Lungo la S.S. 127 Perfugas-Tempio, circa km 57, 15.3.1981, *Corrias & Diana* (SS). — Tempio. Lungo la S.S. 133 Tempio-Palau, circa km 5, 15.3.1981, *Corrias & Diana* (SS). — La Maddalena. Isola di Caprera, valle di Tola, 22.3.1981, *Beltrami & De Logu* (SS). — Padria. Presso il ponte Mannu sul fiume Temo, 22.3.1981, *Corrias & Diana* (SS). — S. Teresa di Gallura. Loc. Tanca di Lena, di fronte la peschiera, 22.3.1981, *Serra* (SS). — Siligo. Bonifica di Paule, km 8, 22.3.1981, *Sanna* (SS). — Giave. Cant. Sa Terralva, 23.3.1981, *Sanna* (SS). — Ardara. Lungo la strada Saccargia-Oschiri, km 14, loc. Pianu Pireddu, 28.3.1981, *Corrias, Diana & Sanna* (SS). — Ozieri. Lungo la strada Saccargia-Oschiri, ca. km 24, loc. casa Lipperi, 28.3.1981, *Corrias, Diana & Sanna* (SS). — Ozieri. Presso il bivio della strada Ozieri-Chiaramonti con la Saccargia-Oschiri, loc. Porcarzos, 28.3.1981, *Corrias, Diana & Sanna* (SS). — Tempio Pausania. Pendici sud di Monte Pulchiana, 28.3.1981, *Sechi* (SS). — Tula. Strada per Erula, dopo il lago, loc. S'Adde, 28.3.1981, *Corrias, Diana & Sanna* (SS). — Tula. Strada per Erula, nel tratto alto sul lago, loc. S. Pietro, 28.3.1981, *Corrias, Diana & Sanna* (SS). — La Maddalena. Guardia Vecchia, 29.3.1981, *Corrias* (SS). — La Maddalena. I. Caprera, bivio per Stagnali, 29.3.1981, *Corrias* (SS). — Luogosanto. S.S. 133 Palau-Tempio, km 30, 29.3.1981, *Corrias* (SS). — Tempio Pausania. S.S. di Palau (n° 133), km 11, 5.4.1981, *Sechi* (SS). — Berchidda. Pendici nord ovest di Monte Figos, 12.4.1981, *Delitala* (SS). — Pozzomaggiore. Lungo la strada per Suni, presso Cantoniera Baddelonga, 16.4.1981, *Corrias & Diana* (SS). — Sindia. Strada Pozzomaggiore-Suni, presso Ponte Badu e Crabolu, 16.4.1981, *Corrias & Diana* (SS). — Suni. Vicino allo stagno Sa Paule, 16.4.1981, *Corrias & Diana* (SS). — Bolotana. Mularza Noa, m 980, 20.4.1981, *Corrias & Diana* (SS). — Bolotana. Punta Palai, m 1200, 20.4.1981, *Corrias & Diana* (SS). — Burgos. Foresta di Burgos presso la Caserma dei Carabinieri, 20.4.1981, *Sanna* (SS). — Tempio Pausania. S.S. 133 per Palau circa al km 6 prima di Cant. Padulo, 20.4.1981, *Sechi* (SS). — Tempio Pausania. Padulo, loc. Conca di Mezu, 21.4.1981, *Sechi* (SS). — Olbia. Berchiddeddu, pendici SE di Monte Ruiu, m 300, granito, 22.4.1981, *Sechi* (SS). — Berchidda. S.S. 199 al km 34, presso il ponte Badu De Mela, 4.5.1981, *Sanna* (SS). — Mandas. All'uscita del paese, 13.5.1981, *Camarda & Sechi* (SS). — Laconi. S.S. 128 al km 74, su calcare, 14.5.1981, *Camarda & Sechi* (SS). — Lodé. Strada Lula-S. Anna, circa al km 28 sotto Punta Su Mutucrone, 17.5.1981, *Corrias & Diana* (SS). — Tempio Pausania. Padulo, 3.4.1982, *Sechi* (SS). — Bolotana. A nord del paese, loc. Ferulas. Basalti, m 600, 18.4.1982, *Diana & Sanna* (SS). — Bolotana. Campeda, lungo la strada per Badde Salighes, loc. Serra e Rughes. Basalti, m 700, 18.4.1982, *Diana & Sanna* (SS). — Bolotana. Strada sotto il costone da P. Oruveda verso il paese. Basalti, m 900, 18.4.1982, *Diana & Sanna* (SS). — Bonorva. Prati lungo la strada per Foresta di Burgos prima delle fonti di S. Lucia, loc. Sa Camba Noa, 18.4.1982, *Villa* (SS). — Aggius, 29.4.1982, *Serra* (SS). — Aggius. Reg. Abba Fritta, 29.4.1982, *Serra* (SS). — Aglientu, loc. L'Impostu, 29.4.1982, *Serra* (SS). — Luogosanto. Funtana Lu Cantaru, 29.4.1982, *Serra* (SS). — Tempio Pausania. Campo Maiori, 29.4.1982, *Serra* (SS). — Tempio Pausania. Cant.

Scupetu, 29.4.1982, *Serra* (SS). — Tempio Pausania. Stazzo Lu Saliciu, 29.4.1982, *Serra* (SS). — Bolotana. Presso il ponte sul Rio Tuilo al km 79,5 della S.S. 129, 2.5.1982, *Corrias & Diana* (SS). — Oliena. Monte di Oliena, pineta artificiale sotto Maccione, 2.5.1982, *Corrias & Diana* (SS). — Laconi. Sarcidano, da S. Sofia a Cant. Ortuabis. Bosco rado a Nord di S. Sofia. Calcare, m 800, 6.5.1982, *Corrias & Diana* (SS). — Meana Sardo. Sarcidano, da Cant. Ortuabis a Laconi. Loc. Doneiddu. Limite tra calcare e basamento, m 820, 6.5.1982, *Corrias & Diana* (SS). — Meana Sardo. Sarcidano, da Cant. Ortuabis a Laconi. Presso il ponte Malifattu. Calcare, m 750, 6.5.1982, *Corrias & Diana* (SS). — Tempio Pausania. Reg. Vallicciola, 16.5.1982, *Serra* (SS). — Meana Sardo. Sarcidano, da Cant. Ortuabis a Laconi, loc. Doneiddu. Limite tra calcare e basamento scistoso, m 820, 27.5.1982, *Corrias, Diana & Villa* (SS). — Meana Sardo. Sarcidano. Prati aridi a sud di Cant. Ortuabis. Calcare, m 800, 27.5.1982, *Corrias, Diana & Villa* (SS). — Guspini. Salendo lungo la strada dalla Miniera al paese di Montevecchio. Scisto, m 350, 1.4.1984, *Villa* (SS). — Tempio Pausania. M. Limbara, Vallicciola. Granito, m 1000, 19.5.1985, *Corrias & Diana* (SS). — Ardara. Strada per Monte Santo. Presso il bivio per Mores. Basalto, 5.4.1986, *Corrias & Diana* (SS). — Laconi. Sull'altipiano sopra la stazione ferroviaria. Calcare, m 600, 12.4.1987, *Corrias & Rossi* (SS). — Tempio Pausania. Monte Limbara, Località Vallicciola. Granito, m 1000, 8.5.1988, *Corrias & Diana* (SS). — Guspini. Salendo lungo la strada dalla Miniera al paese di Montevecchio. Scisto, m 350, 12.4.1989, *Corrias* (SS). — Siliqua. Strada statale da Siliqua a Giba, 12 km a sud di Siliqua al km 43,5, sul bordo della strada presso Campanasissa, Sulcis, 12.5.1989, *Corrias* (SS). — Macomer, Strada Campeda-Bolotana, nei pressi del bivio con la Carlo Felice. Loc. Chercu Arcadu, m 650, basalti, 16.4.1990, *Corrias & Diana* (SS).

As the occurrence of *O. morio* in Sardinia can be ruled out by the evidence given in this study, the locations reported in both figs. 1 and 2 represent an analytical record of the range of *O. longicornu* in this island. This species is widespread all over Sardinia, where it is the commonest orchid species. It can be found in a diversity of habitats (meadows, maquis, wood edges, wood clearings, pastures, borders of roads), from 0 to 1500 m a.s.l. *O. longicornu* appears to be relatively indifferent to the substrate. It flowers from the end of February to May, according to the altitude, microclimatic conditions, and differences in the seasonal course.

Conclusions

From the genetic and morphological data reported above it can be concluded that *O. morio* is not present in Sardinia. The material studied belongs to a single species, that corresponds morphologically to *O. longicornu* from *patria typica* (Algeria). The quotations of *O. morio* from Sardinia appear to be misidentifications of *O. longicornu*, possibly owing to the frequent use in diagnosis of single morphological characters which overlap considerably between the two species.

The finding of diagnostic loci (each giving a probability of correct identification that varies from 0.90 to 1) makes it possible to distinguish *O. morio* and *O. longicornu* at the electrophoretic level. These genetic markers permit the study of the occurrence and the role of natural hybridization and introgression

phenomena between these two taxa in areas where they coexist or come into contact, and from where hybrids were described, such as southern Corsica (CAMUS & CAMUS, 1928) and north-eastern Sicily (ACKERMANN & ACKERMANN, 1988). A genetical and morphological study on hybridization and introgression between *O. morio* and *O. longicornu* in Corsica and Sicily, and on their evolutionary consequences, is in progress (ARDUINO, CIANCHI, BULLINI, ROSSI & CORRIAS, 1989; CIANCHI et al., in preparation).

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Summary

Data are given on the genetic structure, morphology and chorology of the Long-Spurred orchid, *Orchis longicornu* Poiret (1789) from Sardinia and the occurrence in this island of the morphologically similar *Orchis morio* L. (1753), often recorded for Sardinia, is investigated. The genetic analysis of 27 enzyme loci in population samples from locations where both species had been recorded showed that: (*i*) in the population samples from Sardinia the polymorphic loci are in Hardy-Weinberg equilibrium; (*ii*) these samples are genetically poorly differentiated from each other (average Nei's $D = 0.01$): less than 2% of the overall genetic variation observed is attributable to differences between populations ($G_{ST} = 0.015$); (*iii*) a high rate of gene flow was estimated between Sardinian populations: $Nm \approx 4$, possibly owing to seed dispersal by wind; (*iv*) when Sardinian samples are compared with *O. morio* from continental Italy, significant differences in genetic variation were observed: average $H_e = 0.16$ in the former, 0.12 in the latter; (*v*) the genetic distance found between Sardinian populations and those of *O. morio* from continental Italy is relatively high: average Nei's $D = 0.18$, average Rogers' $D = 0.22$; (*vi*) highly significant differences in allele frequencies were found at a number of loci (*Mdh-2, Sod-3, Pgm-1, Gpi-1, Gpi-2*) between populations from Sardinia and continental Italy, each giving a probability of correct identification that varies from 0.90 to 1 (diagnostic loci). These findings provide evidence that *O. morio* is not present in the material genetically analyzed from Sardinia, which includes a single species, apparently corresponding to *O. longicornu*. The slight heterogeneity observed at some loci in the Sardinian population samples can be attributed in some cases (e.g. *Mdh-1, Adh, Est-6*) to local differences, possibly adaptive; in others (e.g. *Pgm-1* and Gpi-*1*) to genetic drift effects. The hypothesis that some alleles (*Gpi-1*^{100}, *Dia*^{107}, *NADHdh-2*^{96}) found in *O. morio* and recorded at low frequency in a few Sardinian populations reflect palaeointrogressive phenomena (owing to sporadical immigrant individuals of *O. morio* having diluted their genes in *O. longicornu* genome through multiple generations of backcrosses) is not sufficiently supported by the available data. The genetic data are in agreement with the results from morphological studies. These involved the examination of about 1500 specimens (both alive and dried) from all over Sardinia, and their comparison with herbarium specimens of *O. longicornu* from *patria typica* (Algeria) and of *O. morio* from continental Italy. The typification of *O. longicornu* is given. The specimens from Sardinia correspond well, on a morphological basis, to the Algerian ones and therefore were all assigned to *O. longicornu*. Several characters were considered, differentiating *O. longicornu* from *O. morio* both in fresh and dried specimens. However, some of them, currently used in diagnostic keys, show much overlap between the two species. This explains the quotations of *O. morio* from Sardinia, that appear to be misidentifications of *O. longicornu*. A description of *O. longicornu* from Sardinia is provided, and its distribution in the island is defined, on the basis of (*i*) a critical revision of literature records, and (*ii*) the examination of fresh and dried Sardinian specimens. *O. longicornu* is shown to be widespread throughout Sardinia, living in many different habitats from 0 to 1500 m a.s.l.