

***Genista thyrrena* Valsecchi (Fabaceae) from Pontine Islands: a real case of importation?**

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Riassunto. *Genista thyrrena* Valsecchi (Fabaceae) delle Isole Pontine: un caso reale di importazione?

Genista thyrrena è una specie endemica delle isole Eolie e Pontine, appartenente ad un gruppo di specie a distribuzione peritirrenica.

È stato intrapreso uno studio molecolare per verificare se abbiano avuto luogo dispersioni a partire da una delle due popolazioni disgiunte di questa entità.

Dai risultati ottenuti confrontando le sequenze plastidiali dell'introne del *trnL*^(UAA) nelle due popolazioni, si evince che vi è stato un fenomeno di dispersione, probabilmente antropogena, dalle isole Eolie verso le isole Pontine.

Key words: Dispersal, *Genista thyrrena*, *Genisteeae*, *trnL*^(UAA)

INTRODUCTION

Genista thyrrena Valsecchi (Valsecchi, 1986) is a species belonging to a group of taxa which has been recently segregated from *G. ephedroides* DC. It is endemic to the Aeolian and Pontine Islands, Italy, and grows on acidic soils of volcanic origin.

Recent investigations on *G. ephedroides* DC. resulted in the fragmentation of the species in various new taxa (Valsecchi, 1986; Maire, 1987; Brullo *et al.*, 1993; Valsecchi, 1993; Brullo & De Marco, 1996). The group, besides *G. thyrrena*, includes the following narrowly endemic Italian species: *G. cilentina* Valsecchi (Campania, Southern Italy), *G. demarcoi* Brullo, Scelsi et Siracusa (Madonie mountains and neighboring area, in Sicily), *G. ephedroides* s.s. (north-eastern Sardinia), *G. gasparrini* (Guss.) Presl (mountains near Palermo, Sicily) and *G. valsecchiae* Brullo et De Marco (south-western Sardinia). Moreover, two other extra-Italian species belong to the group: *G. numidica* Spach (North-Eastern Algeria)

and *G. dorycnifolia* Font Quer (Ibiza, Balearic Islands).

All the above-mentioned segregate species mainly differ in characters related to habit, erectness of the racemes, as well as calyx and corolla shape and size. In particular, according to VALSECCHI (1986), *G. thyrrena* differs from the other species for its “incurved and flexible branches, for the dense multiflowered inflorescence, for the bracteoles and for the main characters of the calyx, stand and legume”.

The distribution of this taxon has been object of various speculations (DE MARCO *et al.*, 1985; DE CASTRO *et al.*, in press). The former authors suggested that the present range of this species is a consequence of more or less accidental anthropogenic importation from Aeolian Islands. The latter authors find that *G. thyrrena* from Ponza has a duplication starting at nucleotide 317 of a 5' - GAT TAA TAA AG - 3' motif in the *trnL*^(UAA) intron. This insertion is not present in *G. thyrrena* accessions from Aeolian Islands or in any other member of the *G. ephedroides* group, and therefore, seems to be unique to the population of the Pontine Islands. Given the presence of the above mentioned duplication, which is an exclusive marker of the accessions from Pontine Islands, DE CASTRO *et al.* (in press) concluded that the species in study is unlikely to have been recently imported from Aeolian Islands.

Regardless, the just mentioned study, dealing with a whole section, forcefully employed few samples per species. To better assess the origin of the population of *G. thyrrena* in Pontine Islands, a molecular investigation was undertaken on the populations of the Aeolian and Pontine Islands using the *trnL*^(UAA) intron. Various specimens have been analysed to observe whether this insertion occurs in all the investigated individuals. Samples from various other species of the *G. ephedroides* complex were also examined. In particular, samples of *G. demarcoi* and *G. gasparrini*, two species are in the same clade with *G. thyrrena* in a previous molecular study (DE CASTRO *et al.*, in press). Finally, samples of *G. cilentina* were also employed, since this taxon in the study by DE CASTRO *et al.* (in press) is at the base of the whole group and, therefore, it may inclu-

de in its genotypes markers which have later segregated in descendant populations/species.

G. thyrrena was reported also for Ventotene. Regardless, after recent repeated botanical explorations, plants have not been found in that island. However, according to ANZALONE & CAPUTO (1974-75) this species was very rare, and clearly introduced in Ventotene, as it was present only as a divide between two fields in the whole island.

MATERIAL AND METHODS

A list of the taxa employed in the present study, together with their origins, is reported in Tab. 1.

The total of examined samples is one hundred and ninety-nine. Each taxon has been sampled in such a way to reflect the actual dis-

Tab. 1 - Taxa, origin and sample size

TAXA	ORIGIN	SAMPLE SIZE (INDIVIDUALS)
<i>Genista cilentina</i> Valsecchi	Palinuro	17
	Caprioli	15
	Pisciotta	16
	Marina d'Ascea (Campania)	17
<i>G. demarcoi</i> Brullo, Scelsi et Siracusa	Ponte Miricola (Sicily)	24
<i>G. gasparrini</i> (Guss.) Presl	Mt. Gallo (Sicily)	24
<i>G. thyrrena</i> Valsecchi	Ponza (Pontine Islands)	50
<i>G. thyrrena</i>	Vulcano (Aeolian Islands)	18
<i>G. thyrrena</i>	Stromboli (Aeolian Islands)	18

tribution on the territory.

DNA was extracted by using Qiagen's Plant DNeasy Mini Kits or according to DOYLE & DOYLE (1990), with the modifications suggested by KÄSS & WINK (1997), i.e., with a lysis for 30 min at room temperature and a phenol/chloroform step.

Chloroplast *trnL*^(UAA) intron was amplified using the two primers reported by TABERLET *et al.* (1991). PCR reactions were carried out following the conditions employed in ACETO *et al.* (1999). PCR amplification products were then purified by using GFX™ PCR DNA Purification Kit (Amersham Pharmacia Biotech Inc.).

By inspection of the *trnL*^(UAA) intron sequences (Genebank accession nos. AJ404463 for Aeolian Islands and AJ404464 for Pontine Islands) obtained in DE CASTRO *et al.* (in press), a restriction endonuclease was found cleaving the fragment in an appropriate way as to make easy the detection of the presence of the insertion. After digestion of the *trnL*^(UAA) intron with *Alu* I restriction endonuclease (Amersham Pharmacia Biotech Inc.), three fragments were produced (168, 250 and 90 bp in presence of the insertion, or 168, 238 and 90 bp in its absence).

Digestion products were electrophoretically separated on a 4% MethaPhor (FMC BioProducts) gel stained with ethidium bromide and photographed on a UV transilluminator. A 100 base pair (bp) ladder (Amersham Pharmacia Biotech Inc.) was used as molecular weight marker, with standards of digested *trnL*^(UAA) intron coming from sequenced individuals of the Pontine and Aeolian Islands.

Some samples have been sequenced in both directions by using a modification of the Sanger dideoxy method (SANGER *et al.*, 1977) as implemented in a double strand DNA cycle sequencing system with fluorescent dyes. Sequence reactions were then loaded into a 373A Applied Biosystems Automated DNA sequencer (Applied Biosystems).

The sequence were aligned using the SEQUENCE NAVIGATOR 1.0.1 software (Applied Biosystems).

RESULTS AND DISCUSSION

As far as the populations of Aeolian Islands are concerned, no one out of the eighteen samples from Vulcano and an equal number of samples from Stromboli has shown the insertion.

Out of the fifty samples coming from Ponza Island, forty-eight individuals showed the insertion and only two (corresponding to 4% of the sample) lacked it. These two individuals were redigested and successively sequenced to verify the absence of the insertion (Fig. 1).

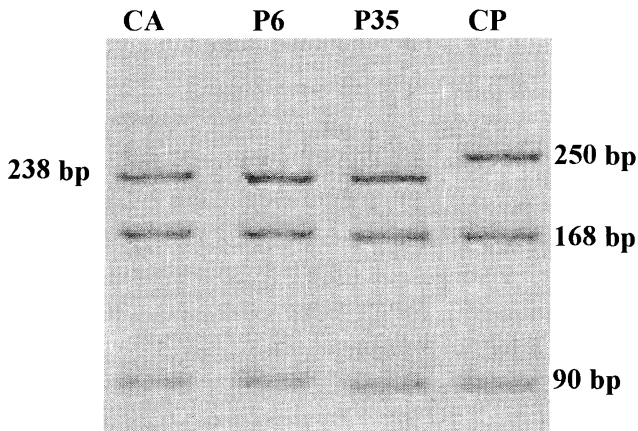


Fig. 1. Example of polymorphic fragments of *trnL*^(UAA) intron digested with *Alu* I. (CA, standard of an Aeolian sample; Ponza's two individuals without insertion; and CP, standard of a Pontine sample)

No specimen from the populations of *G. cilentina*, *G. demarcoi*, and *G. gasparrini* showed the insertion.

The intron sequences, except for the insertion, were identical in all the sequenced specimens of Aeolian and Pontine Islands.

These results may be interpreted by hypothesising that the more frequent haplotype of the Pontine islands generated *de novo* there.

However, whether the two individuals with the other haplotype, the one present in all the other investigated specimens outside Ponza, represent a plesiomorphic condition, or a new, locally gene-

rated haplotype is matter of speculation.

Interestingly enough, the two samples from Ponza Island (4%) lacking the insertion were both collected between the Guarini area and Mount Guardia, immediately close to Ponza's port; the area in which they grow is very steep, and no traces of past cultivation are visible. This area may therefore represent a relic area, comparatively undisturbed, where the plesiomorphic haplotypes are still preserved.

This hypothesis, however, does not justify such a disproportion between the frequencies of the two haplotypes: a neutral marker, as the chloroplast *trnL*^(UAA) intron should be, normally varies its frequencies only by genetic drift in the population, and, in case of a new mutation, one would expect less extreme distributions. On the other hand, chloroplast markers have a tendency to become fixed in the population (DUMOLIN *et al.*, 1995). We, therefore, do not know whether a severe drift occurred (for example, because of a fire in a very small population) or we are now observing the conclusive event of the fixation process. However, in both cases it must be presumed that a casual event allowed distribution to the rest of the island only of the new genotype (where it got widespread because of the abandonment of cultivated land), leaving the primitive one confined to a single area of the island.

On the other hand, the absence of the insertion in the two individuals from Guarini/Mount Guardia may also be interpreted as a loss of the insertion in one individual whose two offsprings have been observed and account for the 4% of the sample. This is almost equally probable as compared to the first hypothesis, and would not require hypotheses of drift or bottlenecks.

In the case of the “plesiomorphic haplotype” hypothesis, however, we may presume that *G. thyrrena* has been recently introduced in Ponza (so that haplotypes are not fixed yet). This would be asseverated by the fact that, according to BÈGUINOT (1905), *G. thyrrena* (called *G. ephedroides* by him) was present only in few areas of the island at the time.

The absence of *G. thyrrena* in Ventotene Island, as already said, may be related to the fact that in the just mentioned island the land

is still widely kept as cultured fields, and, moreover, the territory is much more urbanized than in Ponza. However, as we observed earlier in this paper, the few, introduced, individuals found by ANZALONE & CAPUTO (1974-75), have not been found, and, certainly did not diffuse as in Ponza.

In conclusion, the possibility of dispersal from the Aeolian to the Pontine Islands, which was excluded by DE CASTRO *et al.* (in press), was rejected in the absence of the data presented here, and should be reconsidered. In the light of the present results, in fact, a dispersion event may be hypothesized from Aeolian to Pontine Islands. At this point, no clear statement can be made about the tempo and mode of this colonization; however, the anthropic action may have been decisive. In fact, DE MARCO *et al.* (1985) suggest that this species may have been imported to Ponza for its use as a wind shield, given its arborescent habit.

Acknowledgements. We thank Ms. Ida Sorrentino for technical help in part of this research.

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Abstract

A molecular investigation has been undertaken in order to verify whether dispersion occurred between the disjunct populations of this taxon.

Results obtained by comparing the plastid sequences of the *trnL*^(UAA) intron in the two populations indicate that a dispersion event, probably anthropogenous, occurred from the Aeolian to the Pontine Islands.