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Ecological patterns of morphological variation in Italian populations of *Romulea bulbocodium* (Iridaceae)

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ABSTRACT

Romulea bulbocodium is a steno-Mediterranean species found in widely diverse habitats; it shows considerable morphological variations among different populations. The present study used morphological and numerical analyzes to investigate whether and how different habitats affect Italian populations of *R. bulbocodium* to generate ecological patterns of morphological variation. Individuals from 21 Italian populations in three geo-lithology based habitat types (coastal habitats on calcareous sediments and inland habitats on limestone or magmatic rocks) were sampled in three randomly chosen plots along transects. Morphological, soil chemistry and geographical parameters were measured. The results indicate that morphological variation in Italian populations of *R. bulbocodium* is associated primarily with geographical variables, reflecting climatic and edaphic factors. Sicilian individuals showed strong floral distinctness compared to individuals from other regions, underlining that local phenotypic differentiation is associated with geographic isolation. Furthermore, plants growing in magmatic habitats versus coastal habitats showed the largest morphological distinctness indicating that phenotypes are also influenced by soil chemistry and habitats. These morphological differences are linked to ecological factors such moisture and mineral content of soils. Flower characters that significantly distinguish Italian populations in different habitats has been used by other authors as diagnostic tools to differentiate species of the genus *Romulea* and we can suppose that they are constrained genetically. However, our results do not support the recognition of infraspecific taxa in *R. bulbocodium* but instead sustain the identification of two different ecotypes growing in calcareous coastal and inland magmatic habitats, respectively.

1. Introduction

The genus *Romulea* Maratti belongs to the subfamily Crocoideae (syn. Ixioideae), which includes over 800 species and about 30 genera and is the largest subfamily of Iridaceae (Işik and Dönmez, 2007; Kutbay et al., 2001; Özdemir et al., 2011). According to different authors (Béguinot, 1907, 1909; Čarni et al., 2014; Marais, 1980), the genus comprises about 95 species, most of which are found in southern Africa and the Arabian Peninsula (Manning and Goldblatt, 2001); about 15 species can be found in the Mediterranean basin (Peruzzi et al., 2011). The genus *Romulea* is distinguished from other genera of Iridaceae by its high degree of polymorphism and intraspecific variability (Frignani and Iiriti, 2008). Evolutionary processes hypothesized to be common in this genus are: (i) hybridization, (ii) polyploidy, (iii) tendency to create populations with particular morphological adaptations to climatic and edaphic conditions (Frignani and Iiriti, 2011a). Gynodioecism and sexuality that may be linked to flower size and intensity of perianth color have been reported in the genus (Marais, 1984). In Italy 12 autochthonous species occur (Frignani and Iiriti, 2011a,b,), that are important components of the Italian flora. Although some studies on Mediterranean species of *Romulea* have been published (Čarni, 2014; Erol and Küçüker, 2003; Frignani and Iiriti, 2011a,b; Goldblatt et al., 2002; Işik and Dönmez, 2007; Kök et al., 2007; Özdemir et al., 2011; Peruzzi et al., 2011; Rita, 1989–1990), relationships among Euro-Mediterranean taxa have not been sufficiently investigated and many of the entities described are either subordinated to or considered synonyms of *Romulea bulbocodium* (L.) Sebast. & Mauri

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sensu lato (Frignani and Iiriti, 2011b). On the other hand, there is a synoptic review of sub-Saharan species of *Romulea* by Manning and Goldblatt (2001).

Extensive field studies on *Romulea* in Italy have recently revealed morphological differences among populations of *R. bulbocodium* that occur only a few miles apart but in widely diverse habitats. To our knowledge, no morphological discontinuities have been formally recognized in Italian populations of this species and no previous study has used appropriate objective methods and sampling strategies to identify morphological variations within and among populations of *R. bulbocodium*. Previous studies analyzed karyology (Peruzzi et al., 2011) and few characters from corm tunic morphology (Erol and Küçüker, 2003; Erol et al., 2008), in some Euro-Mediterranean species of the genus.

An effective method for detecting morphological variations is morphometry, widely used in various disciplines including systematics (Asmussen et al., 2006; Gage and Wilkin, 2008; Goldman et al., 2004; Henderson, 2006) along with multivariate methods. An advantage of morphometry is objectivity in data handling, especially when many variables are involved (Manly, 1994). In addition, studies conducted on *Crocus* and *Iris* (Kandemir, 2010; Sapir et al., 2002) showed that morphometric analyzes can be useful in clarifying systematic questions in Iridaceae. Multivariate methods have been widely used as taxonomical tools across taxonomic groups to answer different questions, including ecological influence of geography and soil parameters on morphological variability within taxa (Debussche and Thompson, 2003; Ellison et al., 2004; Pinheiro and De Barros, 2007). Multivariate methods can reveal morphological discontinuities and guide the selection of diagnostic characters (Palestina and Sosa, 2002; Reinhammar, 1995; Tyteca and Dufrêne, 1994), producing results at the phenotypic level that can be tested further at the genotypic level.

In this study we investigated whether different habitats host Italian populations of *R. bulbocodium* morphologically different, generate ecological patterns of morphological variation. We also evaluated patterns in relation to geography by examining morphological variability across the geographical range of the species.

The main hypothesis, tested by statistical analysis of morphological data from individuals sampled directly in the field, was that individuals of *R. bulbocodium* from different habitats, chosen *a priori* on the basis of the geo-lithological nature of their substrata, were significantly distinct in morphological characters and that the differences were linked to soil chemistry. The alternative hypothesis was that morphological variations of this geophyte do not reliably correlate with variation observed in soils of different habitats.

We addressed two main questions about Italian populations of *R. bulbocodium*:

- Are the observed morphological variations associated with habitat, soil chemistry or geographic gradients?

- Are the observed patterns of morphological variation in agreement with identification of phenotypes/ecotypes based on previous morphological analyzes?

2. Materials and methods

2.1. Biological materials

Romulea bulbocodium (L.) Sebast. & Mauri is a bulbous geophyte, 3–15 cm tall, with floral stem having 1–3 flowers. Basal leaves are 2, short, sheathing while stem leaves 3–5, subcylindrical, linear (1–1.5 mm wide). Tepals are elliptic, sharp, yellow to the base, white in the intermediate part, violet in the rest. Stamens are long about 1/3 of perianth and the style is frequently longer than anthers. The capsule is ellipsoid, with subspherical brownish seeds. The species has a steno-Mediterranean distribution and is common in the Mediterranean basin, although populations are localized and limited in size. *R. bulbocodium* is one of the most variable species of the genus, and among Italian species of *Romulea*, has the largest geographical and ecological range. In Italy, it can be found in many habitat types, such as behind dunes along the Tyrrhenian coast, in woodland clearings and open areas on magmatic substrates and in arid limestone grasslands. Béguinot (1908) observed the complexity of *R. bulbocodium*, reporting fragmentation suggesting several taxa, often interpreted as species but probably best recognized as varieties. More recently some authors acknowledged intraspecific variability within its distribution (Marais, 1980; Pignatti, 1982), while its complexity was indicated by ten described subspecies and nine described varieties (Barker and Govaerts, 2011).

2.2. Sampling design and field collection

A previous investigation based on herbarium specimens deposited in eight Italian herbaria (Erbario Dipartimento di Scienze Botaniche e Orto Botanico Università degli Studi di Cagliari, CAG; Herbarium Plant Biology Section Department of Biology "Marcello La Greca" Università di Catania, CAT; Herbarium Universitatis Florentinae, FI; Herbarium Horti Pisani, PI; Erbario Dipartimento di Biologia Vegetale Università degli Studi di Roma La Sapienza, RO; Erbario Dipartimento di Scienze del Farmaco Università di Sassari, SASSA; Herbarium Universitatis Senensis, SIENA; Erbario Dipartimento di Biologia Università degli Studi di Trieste, TSB; Erbario Dipartimento di Botanica ed Ecologia Vegetale Università di Sassari, SS; Erbario della Tuscia, UTV) showed that *Romulea bulbocodium* is mostly distributed in central Italy, Sicily and Sardinia. Using herbarium specimens it was possible to identify 80 sites, but only 47 were based on observations since 1950, where the chances of finding the species were higher. The geo-lithological character of these sites was obtained from data on the Geoportale Nazionale (2007), making it possible to group the sites into three types of habitats (abbreviations in brackets): (i) coastal habitat on calcareous sediments (CC); (ii) inland habitat on limestone (IL); (iii) inland habitat on intrusive (granitoid) and effusive (lava and pyroclastic) magmatic rocks (IM).

For each of the three habitats, seven sites were randomly selected for a total of 21 sites (approximately 50% of the collection sites of herbarium specimens since 1950). These sites were distributed across Italy: 12 in central Italy, five in Sicily and four in Sardinia. Plant material was obtained during field collections at these sites in spring 2012. Progressive blooming at different latitudes allowed us to concentrate sampling in a single season, avoiding analysis of samples from different surveys that could respond differently to season. At each site we found a population of R. bulbocodium growing in a regular-shaped polygon. Along the major axis of the population, estimated visually, we placed a transect along which we randomly chose three plots of 1 m × 1 m. A total of 63 plots (3 plots × 21 sites) were sampled, collecting one plant in bloom from each plot. When a plot contained more than one plant, we sampled the most central plant in the plot; if no plant was present, another plot was chosen.

In each plot we recorded the following parameters: GPS coordinates, altitude (m a.s.l.) and bioclimate using the map of Italy (Blasi and Michetti, 2007). Six bioclimates were found in our sites, to which we assigned increasing numbers from hot–dry (1) to cool–wet (6) corresponding to following bioclimates: 1– Mediterranean oceanic; 2 – Mediterranean oceanic-semicontinental; 3 – Mediterranean oceanic (transitional, subhumid); 4 – Mediterranean oceanic (transitional, humid/subhumid); 5 – Temperate oceanic-semicontinental; 6 – Temperate semicontinental-oceanic (transitional).