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Mate limitation in populations of the endangered *Convolvulus lineatus* L.: A case for genetic rescue?

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ABSTRACT

In self-incompatible clonal plants, the spread of individual plants can exacerbate mate limitation to the point that it becomes a serious constraint on long-term population persistence, especially in small, isolated populations. In such species, it may be necessary to introduce new genetic material from other populations to restore seed production, a strategy termed "genetic rescue". In this study we assess the potential pertinence of such genetic rescue in the clonal perennial plant *Convolvulus lineatus* L., whose populations are often highly reduced in spatial extent and are currently being fragmented by land development projects in Mediterranean France. To do so, we quantify fruit production in a range of populations of different size over four years and perform a series of hand-pollination experiments in natural populations to assess whether fruit set is limited by mate availability. We found that *C. lineatus* is a self-incompatible species that shows extremely low values of fruit set in natural populations and that a principal cause of this low fruit set is a lack of compatible pollen. This may be primarily due to clonal spread that causes individual populations to be comprised of patches containing one or very few incompatibility types. In small populations fragmented by human activities and which show an absence of fruit production, we thus argue that genetic rescue represents a promising conservation management strategy to avoid inevitable long-term future population decline. We discuss how best to introduce new genetic material into the study populations.

Introduction

Pollen limitation, a widespread phenomenon in plants, is characterised by a decrease in plant reproduction due to inadequate pollen transfer among potential mates (Ashman et al. 2004; Knight et al. 2005; Larson & Barrett 2000). Pollen limitation can be caused by a quantitative lack of pollen or a qualitative lack of compatible pollen. Quantitative pollen limitation can result from deficient pollen transfer among plants (Aizen & Harder 2007) as a result of a diverse range of potential causes, including distance among individuals, barriers to pollen flow and lack of pollen vectors. Qualitative pollen limitation implies reduced transfer of compatible pollen due to high rates of self-pollination or pollination among individuals of the same incompatibility type (Amat et al. 2011). This can cause inbreeding depression in self-compatible plants (Iwaizumi & Takahashi 2012) or reduced fruit set in self-incompatible (SI) plants (Amat et al. 2011; Vallejo-Marin & Uyenoyama 2004). Since pollen vectors typically disperse pollen locally, in SI plants the degree of relatedness between parents may limit seed production as much as insufficient pollination (Aizen & Harder 2007; Harder & Aizen 2010). In the latter case, pollen quality limitation is equivalent to mate limitation (Busch & Schoen 2008).

In self-incompatible plants, mate availability may severely limit seed production when a decrease in population size is associated with loss of genetic diversity at the self-incompatibility locus (Pickup & Young 2008). Loss of diversity and S-alleles (i.e. those which determine the self-incompatibility system) is particularly pertinent to SI clonal plant species in which the spread of individual plants can be rapid and extensive (Cook 1983; Pfeiffer 2007) and cause individual patches (even entire populations) to have limited genetic diversity (Aspinwall & Christian 1992; Wolf et al. 2000a) and even a single clone. As a result individual populations may be dominated by a very small number of individuals with limited S-allele diversity, reducing the possibilities for outcrossing (Charpentier et al. 2000; Vallejo-Marin et al. 2010) and causing pollen discounting in obligate outcrossers (Amat et al. 2011; Charpentier 2001). A critical issue here is that mate limitation and naturally low levels of sexual reproduction may become

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particularly apparent and problematic for clonal species which occur in habitats with a fragmented spatial structure, as a result of either the natural patchy occurrence of their habitat (e.g. see Charpentier 2001; Wolf & Harrison 2001) or extensive human-induced effects on the surface area and spatial configuration of natural populations (Aizen et al. 2002; Weekley et al. 2002; Wolf & Harrison 2001). In such cases, reproductive success may become greatly reduced (perhaps to zero) in individual populations, inciting conservation action.

Genetic rescue, i.e. the introduction of unrelated genes from other populations (Tallmon et al. 2004), is potentially an important means for the conservation of small populations with low levels of genetic diversity. In populations with low genetic variation, the introduction of unrelated individuals can have rapid positive effects on fitness, which go beyond those attributed to their demographic contribution (Newman & Tallmon 2001; Willi & Fischer 2005). Immigrants can cause increased fitness of offspring due to mating among genetically divergent individuals (heterosis) and rapidly spread if they have an adaptive advantage as a result of frequency-dependent selection on rare alleles (Tallmon et al. 2004). Immigrant pollen (potentially with novel S-alleles) has been shown to increase reproductive success in small populations of several SI plant species (DeMauro 1993; Tallmon et al. 2004 and references therein). For example, in a small population of the rare self-incompatible lakeside daisy (*Hymenoxys acaulis* var. *glabra*), an endemic plant unable to reproduce sexually due to the presence of a single mating type (S-allele), introducing pollen from distant populations was necessary for seed set to occur (DeMauro 1993). Hence, recovery protocols should integrate the need to maximise the probability of sampling different S-alleles from multiple source populations in order to restore outcrossing potential within populations. Increasing the number of mating types can thus represent an important means of genetic rescue in plants, which may locally have lost their reproductive potential.

In this study we assess the pertinence of a genetic rescue strategy for *Convolvulus lineatus* L., a listed species in France, whose populations continue to suffer destruction and fragmentation as a result of development projects and high frequentation by motorised vehicles. Many populations are greatly reduced in spatial extent (occupying less than 200 m^2). This species shows extensive clonal growth by rhizome extension and it is impossible in the field to distinguish individual plants. Isoenzyme studies in the closely related clonal and SI *Convolvulus arvensis* have shown that an individual clone can be from 1.5 m to 3 m in diameter (Bernal Velasquez 1985). In *C. lineatus* some populations are composed of a single or only 2–3 patches of plants, each patch containing a variable number of rosettes most of which can be seen to emerge from an underground rhizome. Although plants flower profusely, preliminary observations have indicated that populations in southern France produce very few fruits. This low fruit set could result from the clonal growth of the plant and may occur even in spatially large populations. What could be problematic is that in small and highly fragmented populations this low fruit set may be reduced to zero as a result of total loss of potential mates.

The objectives of this study are to (1) determine whether *C. lineatus* is a SI species, (2) precisely record fruit production in a range of populations of different spatial structure and extent over several years, and (3) test for limited mate availability by performing supplementary hand pollinations using pollen donors in the same and different populations. We integrate into the study a population that has been "protected" from land-development projects in a fenced enclosure in order to assess the pertinence of a conservation strategy based on protecting individual small populations for this species.

Methods

Study species

C. lineatus (Convolvulaceae) is a rhizomatous perennial herb, with linear pubescent glaucous leaves, flowers with a radiate white or pink corolla and capsules with 1–3 seeds. It has a mainly Mediterranean distribution, inhabiting dry calcareous Mediterranean garrigues and rocky pastures. It also occurs on the edges of rocky paths and small scree slopes and can colonise disturbed stony areas. The species reaches its northern distribution limits in France, where it is rare and listed for protection in four administrative regions (Provence-Alpes-Côte d'Azur, Auvergne, Pays de la Loire and Poitou-Charentes) (Tela-Botanica 2012). It is more commonly found in habitats close to the coast in the Languedoc-Roussillon region and on Corsica. Flowers are visited by small solitary bees, syrphid flies and small butterflies (R. Berjano pers. obs.). Rhizomes assure vegetative propagation, and plants often form small to large, probably clonal, patches. In some populations there is a single patch sometimes only around 25 m^2, and in many others the population covers < 200 m^2. In southern France, there are several records of populations that once occurred in what are now developed areas. In addition, these areas of intense human activities and land-development surround many current populations. Most natural populations are now highly fragmented, some as a result of natural causes, many as a result of fragmentation due to human activities.

Study sites

We studied seven populations located in France (Fig. 1): Puy Long (PL: 45.898° N; 3.266° E), the only known site of the species in the Auvergne region (Fig. 1A); Sète (ST: 43.528° N; 3.654° E), Gruissan (GR: 43.149° N; 3.064° E) and La Palme (LP: 42.972° N; 2.953° E) in the Languedoc-Roussillon region (Fig. 1B); and Sollac (SL: 43.496° N; 5.009° E), Massilia (MS: 43.603° N; 4.908° E) and Pourra (PR: 43.508° N; 5.020° E) in the Provence-Alpes-Côte d'Azur region (Fig. 1B). Populations differ in spatial extent: ranging from small populations represented by a single large patch covering < 50 m^2 (MS and PR) to more extensive populations (GR and LP), albeit patchy, with a spatial occupancy > 1000 m^2. Intermediate populations between these extremes (ST and SL) occupy between 100 and 300 m^2. The SL population contains two patches with which begin flowering at different dates and show difference in floral morphology.

Of particular importance to our study is the MS population. As part of measures to avoid the impacts of land development on the natural habitat (steppic grassland unique in France) a population of *C. lineatus* has been protected from destruction in a fenced enclosure. This was carried out in the absence of any information on the long-term viability of the species at this site and the potential effects of such development on its persistence. At this site, industrial development since 2009 has led to destruction of natural habitat around the population to the extent that it is now completely surrounded by warehouses.

Experimental design

Natural reproductive success

From 2009 to 2012 we estimated population reproductive success in six of the study populations (ST, GR, PL, SL, MS and PR). The LP population was not included in the study of natural reproductive success because it was discovered only recently after the onset of our study of other populations. In each population, six permanent quadrats of 0.5 by 0.5 m were established, two quadrats with a low ramet density, two with medium density and two with high ramet density. Each quadrat was subdivided into 100 cells of 5 by