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Re-establishment trials in endangered plants: A review and the example of *Arenaria grandiflora*, a species on the brink of extinction in the Parisian region (France)[1]

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Abstract: The successful restoration of plant species in the wild depends on knowledge of the species' habitat requirements and genetic, demographic, and ecological traits that may increase vulnerability to stochastic extinction processes. A few studies have reported experimental projects that attempted to re-establish populations of endangered plants; however, their experimental designs often led to ambiguous results. This paper reviews the common practices in plant restoration and re-establishment programs and reports on an experiment that we performed on an endangered plant (*Arenaria grandiflora*) in the Parisian region. Our aim is to provide advice on how a restoration experiment should be conducted in order to maximize its success.
Keywords: breeding system, monitoring, reinforcement, reintroduction, restoration.

Résumé : Peu de projets de restauration de plantes rares ou menacées figurent dans la bibliographie scientifique. Généralement, les expérimentations se font de manière empirique et ont un taux de réussite assez faible. En effet, pour qu'un plan de restauration ait de bonnes chances de succès, il convient de bien connaître les besoins des espèces en terme d'habitat ainsi que de prendre en considération les caractéristiques génétiques, démographiques et écologiques qui sont susceptibles d'augmenter la vulnérabilité de leurs populations face aux processus stochastiques d'extinction. Dans cet article, nous présentons, à tous les stades des expérimentations, les pratiques utilisées et décrites dans la littérature scientifique. Nous exposons également, à titre d'exemple, l'expérimentation que nous avons menée sur une plante menacée de la région parisienne (*Arenaria grandiflora*). Le but de cette publication est de fournir des conseils sur la manière de conduire une opération de restauration de population en maximisant les chances de réussite.
Mots-clés : réintroduction, renforcement, restauration, suivi, système reproductif.

Nomenclature: Kerguelen, 1993.

Introduction

To date, only a few studies have reported re-establishment trials in plant species. Empirical experiments are rarely preceded by demographic and genetic studies and are often conducted without controls. In such cases, the results are ambiguous. Hence, the use of appropriate biological tools and thorough ecological understanding is essential (Clark & Cragun, 1994).

Three types of restorations can be defined. (1) *Reintroductions* are transplantations of individuals in sites where the species went extinct. (2) *Enhancement* or *reinforcement* refers to the addition of individuals to extant populations, with the aim of restoring their viability through increased population size and/or genetic diversity (Falk *et al.,* 1996). (3) *Introductions* are creations of new populations in sites at which the species had never been reported before, with the aim of restoring the species' viability through an increase in the number of populations or through increased migration between extant populations. Thus, for example, if some populations are too isolated, the new populations among them can constitute "relay" populations that allow gene flow between them. Although each restoration attempt is unique, pursuing certain general rules is required.

Restoration experiments may be generally easier to conduct with plants than with animals, for 2 main reasons.

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First, many plant species are easily multiplied *ex situ* via seeds, cuttings, or *in vitro* culture, enabling the experimenters to obtain the many individuals required for a restoration experiment in less time and with less effort and use of space than is often needed for mammals or birds, which represent the great majority of animals addressed in conservation plans. Second, because plants stay at the same place from the seedling to the reproductive stage, marked or mapped individual plants are easier to survey than animals, which require complicated capture–mark–recapture designs. Moreover, survival estimates are generally more accurate in plants than in animals. (However, for plants with dormancy, capture–recapture methods have been proven to be the most suitable for estimating plant dynamics [Kéry, Gregg & Schaub, 2005]). On the other hand, genetic analyses are required in order to estimate gene flow via seed and pollen dispersal. Moreover, many plant species may also reproduce vegetatively, which leads to additional difficulty in determining and identifying an individual (Menges, 1991).

However, the successful restoration of a rare plant species in the wild depends mainly on knowledge of the habitat requirements of the species, which enables identification of suitable restoration sites, and on consideration of key genetic, demographic, and ecological traits that affect vulnerability to stochastic extinction processes (Menges, 1991).

Restoration programs are often based on existing biological methods, and in return they provide data for the validation of theoretical models. Population Viability Analysis (PVA), for example, is a tool that predicts the probability of persistence of a population over a specified duration, given certain demographic events (Bowles & Whelan, 1994), and provides demographic parameters involved in population sustainability (Beissinger & McCullough, 2002). Although PVA is a powerful tool, appropriate and sufficient data sets are often difficult to obtain: (1) PVAs use mid-term (4–5 y) to long-term data sets, and therefore the establishment of experimental monitoring is necessary; (2) study of a large number of populations is required, which is rarely possible for rare plant species; and (3) there are inherent difficulties in generating numbers for complex parts of the plant's life cycle such as dormancy and the occurrence of seed bank (Menges, 2000).

Establishment of long-term viable populations can also be viewed in terms of metapopulation theory. Metapopulations are sets of interacting subpopulations, linked by gene flow and subjected to extinction and recolonization patterns. The fate of each population is influenced—in various proportions—by its genetic, demographic, and environmental traits. Metapopulation persistence depends upon the balance between establishment rates and population extinction rates (McEachern, Bowles & Pavlovic, 1994). For example, in *Proclossiana eunomia*, extinction risk is mainly sensitive to demographic parameters (Schtickzelle & Baguette, 2004) and to environmental stochasticity (Sawchik *et al.,* 2002). In each case, in addition to the position of the deme, there is a minimum deme size and a minimum deme number under which the viability of the metapopulation is unlikely (see, for example, the study of Brito & Fernandez, 2002 on *Micoureus demerarae*). The sustainability of these demes is also influenced by the migration rates between them (Bouchy, Konstantinos & Couvet, 2005).

In many cases, calculations of metapopulation viability parameters could provide guidelines for rare species management, but they are rarely used for this purpose.

Generally, powerful models including demography and genetics are developed in which extinction probability is calculated from factors such as effective population size, heterozygosity rate, number of deleterious alleles, and probability of various stochastic events such as environmental or demographic variations (see Robert, Couvet & Sarrazin, 2007). Different management scenarios can be tested by simulation.

The aim of this paper is to review the common practices used in restoration programs. The experiment we performed on an endangered plant (*Arenaria grandiflora*) in the Parisian region will be presented as an example.

Restoration plans in France

In France, the plant species that have been subjected to re-establishment trials are plants that are considered to be endangered in France, regionally or nationally. The Red List of species threatened at the national level was established in 1982 (list revised in 1995) and contains 486 plant species. The species protected at the regional level are considered to be endangered locally, although they are more common elsewhere in France. In the Parisian region, for example, no endemic plant species exists anymore.

Protected species cannot be destroyed, cut, harvested, rooted up, used, sold, or bought (departmental order of 08/31/1995 published in J.O. 10/17/1995) (Arnal, 1996). Special government permission is required in order to conduct re-establishment trials of protected species. Obtaining authorizations is a long and difficult process, especially when the reinforcement plant material is obtained from a distant source population. Such obstacles may explain why there are so few trials officially conducted in France.

Nevertheless, a variety of re-establishment plans have been implemented, especially by natural-site managers. Methods have frequently been as simple as possible, involving (ir)regular counting of the individuals (before and/or after management) and rough estimation of the breeding system and ecological needs of the plants, but lacking population genetic analyses. Consequently, these restoration actions have rarely led to scientific publication. Examples can be found in a special issue of the Bulletin de la Société Botanique du Centre-Ouest (1999), the proceedings of a meeting on endangered plants of France. Aboucaya *et al.* (1999), for instance, transplanted 5 endemic priority species in the wild in Corsica. These were *Aconitum napellus* subsp. *corsicum*, *Anchusa crispa*, *Herniaria latifolia* subsp. *litardierei*, *Naufraga balearica*, and *Silene velutina*. In addition, Jarri (1999) reinforced 1 population of *Vaccinium oxycoccos* in western France; however, his attempt did not result in a full species recovery.

To our knowledge, only 4 restorations have been performed following preliminary population biology studies.

The first was the introduction of 2 populations of *Centaurea corymbosa* in southern France in 1994. Both the introduced and the natural populations have been regularly monitored since the introduction (Colas, Olivieri & Riba, 1997). Results have shown higher survival in the introduced populations but lower fecundity, caused by the lack of compatible pollen. This issue could have been avoided by sowing more seeds in subsequent introductions in order to enhance the density of flowering plants (Kirchner *et al.,* 2006). Second, in 2002 endangered *Spiranthes spiralis* plants were removed from one of their natural sites for field management in the Fontainebleau forest near Paris. The population was divided into 2 parts: one was reintroduced into the site after fieldwork, and the other was introduced into a new site (in the same forest) as a backup population. This restoration plan was carried out following ecological, genetic, demographic, and reproduction studies (Machon *et al.,* 2003). Five years after the initial restoration, the 2 populations are still in constant increase. The third example is restoration of horsetail (*Equisetum variegatum*) (Machon *et al.,* 2001b) by cloning and transplanting 9 plants in the wild in 2006. Finally, there is the restoration study on *Arenaria grandiflora* that was conducted in the Parisian region, which is discussed in detail below.

Recommended preliminary studies

Successful restoration policy involves 3 criteria that are crucial for the persistence of a species at a given place:

(1) Consistency between the environmental characteristics of the restoration site and the ecological needs of the species;

(2) Sufficient population size to avoid demographic stochasticity or Allee effect problems; and

(3) Sufficient genetic variability to face environmental changes and avoid inbreeding depression.

The latter 2 criteria may, however, be affected and determined by the breeding system of the plants. Similarly, the choice of the monitoring protocol associated with the restoration action will mainly depend on the life history traits of the species studied.

DEMOGRAPHIC AND GENETIC CONSIDERATIONS

Demographic monitoring records the fate of individual plants in a population through repeated *in situ* measurements (Pavlik & Barbour, 1988). To design and implement re-establishment actions, demographic information is essential for 3 purposes: (1) to identify which populations are declining or unstable and thus require management; (2) to determine which life history stages are more critical and focus on them to enhance survival, reproduction, and long-term population vigour (Fernster & Dudash, 1994); and finally, (3) to provide an experimental framework for the evaluation of the restoration efficiency (Harvey, 1985; Given, 1994).

Harper and White (1974) outlined methods for studying plant population demography. However, for many plant species, determining "individual plants (units)" of the populations is problematic. Determining a meaningful and convenient unit depends on the purposes of the study and the morphology and biology of the plants. In species that reproduce only by seeds (annual plants), the individual is the plant that arises from a seed. In species with vegetative reproduction, it is the rooted ramet. The non-rooting ramets capable of producing flowers and seeds can also be recorded as individuals (Bradshaw & Doody, 1978).

Population viability depends on the longevity of individuals and the ability of the populations to replace their members from seeds and/or by vegetative production of new units. The growth rate of the population has 2 components: one associated with seed production and the other associated with vegetative reproduction. Therefore, it is also important to determine population cycle at the beginning of the study. The seed bank in the soil also should be taken into account (despite the technical difficulties) as it may constitute a stock of potential individuals (Doak, Thomson & Jules, 2002).

For plant species, the efficiency of natural pollen transfer is distance dependant (Weller, 1994), a factor that should be considered when determining an appropriate population density in a restoration program. Furthermore, plants that have animal pollination need a minimal population density in order to attract pollinators. This phenomenon is called the Allee effect (see Deredec & Courchamp, 2007). Pollinator-dependant taxa often experience reproductive failures if pollinators are ineffective or absent (Kearns *et al.,* 1998). Oddly, despite the increasing number of publications reporting studies on Allee effects in plant species, restoration plans based on these studies are lacking.

Genetics also contributes to re-establishment studies in many ways (Loeschcke, Tomiuk & Jai, 1994; Schwartz, Luikart & Waples, 2007). Conservation genetics uses, for example, neutral markers to describe genetic organization among and within populations and thus infer the demographic history of the populations. The study of quantitative traits (usually in common garden experiments) allows assessment of the adaptive potential of the remnant populations. Because they are not necessarily linked, the joint study of neutral markers and quantitative traits can provide guidelines for the recovery of small isolated populations.

Extinction probability is often increased in small populations because genetic drift contributes to the loss of genetic variation and the fixation of deleterious alleles. Mating among relatives also leads to reduction in the population's vigour through inbreeding depression. In this context, the aim of genetic manipulations is to restore a population's long-term vigour (Riggs, 1990).

Inbreeding depression can have 2 genetic bases: dominance and overdominance (heterosis), and the role of these 2 possible processes will determine the long-term consequences of inbreeding depression. If dominance is the cause of inbreeding depression, it is expected to be greater in outcrossing than in selfing populations, because there are naturally fewer purges of deleterious alleles (Falconer, 1981). For allogamous species, a controlled breeding program before re-establishment may have the same effect. If the cause of inbreeding depression is overdominance, on the other hand, artificial selection will not be as efficient (Fernster & Dudash, 1994). However, Allendorf and Ryman (2002) underline the difficulty of modeling inbreeding depression in natural populations.

In any case, mixing populations is a solution that should be seriously considered, although precautions have to be taken to avoid the 2 types of risks linked to population translocation: (1) problems of maladaptation at the restoration sites and (2) outbreeding depression (Fernster & Dudash, 1994). Individuals can be highly adapted to their natural environment. When re-establishment actions involve translocating individuals across geographic ranges, the translocated individuals may introduce maladapted genotypes into the new environment. Outbreeding depression occurs when the mating of individuals from distant source populations results in progeny with reduced vigour (for example, Sipes & Tepedino, 1995; Fischer & Matthies, 1997; Quilichini, Debussche & Thompson, 2001). The causes of outbreeding depression are (1) the consequences of alleles selected for their individual effect with hybrid offspring that are not adapted to their parental environment and (2) the breakup of co-adapted gene complexes (Price & Waser, 1979; Waser, 1983; Templeton, 1986; Waser & Price, 1989; Lynch, 1991; Fischer & Matthies, 1997; Affre & Thompson, 1999; Quilichini, Debussche & Thompson, 2001).

Re-establishment plans require a balance between maximizing genetic diversity, helping to purge deleterious alleles, avoiding breaking local co-adapted gene complexes, and avoiding importation of maladapted genes. Mixing populations may be useful if heterosis and the appearance of adaptive novel genotypes outweigh inbreeding depression (Templeton, 1986).

The origin of introduced individuals is thus a key issue. Individuals are more likely to be adapted to a site in which they are to be introduced if they originate from the same site and have been away from it for only a short *ex situ* conservation period or if they come from a population connected by gene flow with the population to be reinforced. If such individuals are not available, the safety-first principle recommends introducing plants from habitats as ecologically similar as possible to the habitats concerned with the project, *i.e.,* individuals from differentiated populations and/or adapted to different evolutionary lineages should not be mixed. The concept of "conservation units" emerged in this context (Moritz, 1994), and more recently "exchangeability" was defined as a criterion for determining conservation units (Crandall *et al.*, 2000). Crandall *et al.* (2000) defined 2 types of exchangeability, genetic and ecological, on 2 timescales, historical and recent. He identified rules corresponding to each case of exchangeability between 2 populations, enabling managers of a restoration plan to know if individuals can be translocated/planted from one population to another. Many other methods to define conservation units, in particular Evolutionary Significant Units (ESUs), have emerged recently, generating lots of debate among scientists. Fraser and Bernatchez (2001) have reconciled these various approaches, emphasizing that the choice of a method depends on the studied species and populations.

Breeding system

The development of recovery programs for rare and endangered species requires knowledge of breeding systems (Hamrick *et al.*, 1991; De Mauro, 1993; Weller, 1994). The choice of which individuals to add to maintain viable populations depends on the way the plants mate. However, very little is known about breeding systems in rare or endangered plants (Schemske *et al.*, 1994). Thus, it would be very useful to define protocols for determining the breeding system of the plants when populations are to be manipulated. Breeding system is mainly determined by floral traits and flower gender. Although unisexual flowers exist within families or orders, hermaphrodism is most common among the flowering plants today (Richards, 1996), and many angiosperms have evolved breeding systems that are a compromise between cross- and self-pollination. A short-term approach to determining the breeding system includes observation and quantification of floral design (characteristics of individual flowers), floral display (number of open flowers on a plant and their arrangement within and among inflorescences), and the distribution of flower gender among individuals (Weller, 1994). A longer term approach would use experimental pollination to enable quantification of seed sets for different degrees of selfing and outcrossing. This approach can determine if the species is self-compatible or not and provide an idea of the "optimal crossing (genetic or geographical) distance" in terms of reproductive success. If a species is hermaphroditic, the existence of a self-incompatibility system and its type might be tested.

The species that reproduce mainly vegetatively are easier to restore because regular replacement of the disappearing genotypes is simple, although the populations are not necessarily viable over the long term. Moreover, dioecious species, in comparison to hermaphroditic species, require transplantation of more individuals and can utilize breeding programs more similar (*e.g.,* in terms of sex ratio) to those described for animals.

Populations of allogamous species may persist via vegetative growth for a considerable time, but if the populations are not viable over the long term, it is necessary to promote the conditions (*e.g.,* improvement of habitat quality, supplementation in pollen, or increase of density in plants) that will allow the species to recover sexual reproduction.

The re-establishment of self-incompatible species is difficult compared to restoration of selfing species, especially if self-incompatible populations are so distant that pollen exchange and seed dispersal among them is unlikely. In particular, finding compatible mating types for sporophitic incompatibility systems is essential in the creation of populations (De Mauro, 1993; Young & Brown, 1999; Weekley & Race, 2001). Considerable attention must be paid to heteromorphic species because of the reduced number of effective reproducers. According to Weller (1994), floral traits that promote heteromorphic self-incompatibility, *e.g.,* distyly, tristyly, or dioeciousness can increase population vulnerability to stochastic risks and are often associated with low seed production. For example, for a distylous population, a large number of the 2 different morphs must be employed to re-establish an efficient reproductive population.

Finally, certain species, such as self-incompatible monocarpic species, are particularly difficult to restore, because individuals flower only once, and thus obtaining mature plants can take a long time (at least 3 y after germination for *Centaurea corymbosa* [Asteraceae]; Colas, Olivieri & Riba, 1997; Freville *et al.,* 1998; Kirchner *et al.,* 2006 [Colas, Olivieri & Riba, 2001]). Furthermore, when these plants flower, they have to be synchronous with compatible pollen donors. Hence, creating populations of such species requires many individuals enable to reproduce every year, and so a large number of new genotypes must be translocated.

Experimental design

AIM OF RE-ESTABLISHMENT TRIALS

The goals of re-establishment trials are to enhance population numbers, to reduce the likelihood of extinction from natural random events (Cully, 1996; Gordon, 1996; McDonald, 1996), or to test factors that could favour establishment of rare species (Pavlik, Nickrent & Howald, 1993; Morgan, 1999). Other studies sometimes aim to reintroduce a species where it had occurred before but became extinct (Bowles & McBride, 1996; Guerrand, 1996; Rich, Gibson & Marsden, 1999) or to remove as many plants as possible from a destruction site to a site where the species was nearly extinct (Brumback & Fyler, 1996).

PLANT MATERIAL

The plant material used for reintroduction or reinforcement depends on the biological characteristics of the species and the available material. Different stages of the plant life cycle can be employed in order to avoid the most vulnerable phases. The most common methods used are transplantation of plants obtained by cuttings from natural or garden plant populations (Cully, 1996; Gordon, 1996; Hogbin & Peakall, 1999; Krauss, Dixon & Dixon, 2002) or seedlings from seeds harvested in natural populations and sown in gardens or greenhouses (Guerrand, 1996; McDonald, 1996; Bowles & McBride, 1996; Robichaux, Friar & Mount, 1997; Morgan, 1999). In rare cases, whole plants are moved from their natural site to the reintroduction site (Brumback & Fyler, 1996). For some species, seeds are directly sown in new sites (Pavlik, Nickrent & Howald, 1993; Colas, Olivieri & Riba, 1997; Milton *et al.,* 1999; Rich, Gibson & Marsden, 1999; Colling, Matthies & Reckinger, 2002), which reduces the risks of soil translocation and transplantation stress for the plants. However, establishment success from seeds is often low (sometimes less than 1%; Milton *et al.,* 1999). To provide seeds to establish new populations, the number of seeds removed from natural populations has to be high, increasing extinction risk (Menges, Guerrant & Hamzé, 2004)

The number of translocated plants ranges from a few individuals (25 seedlings of *Stryrax texana* [Styracaceae]; McDonald, 1996) to thousands of plants (thousands of *Filago gallica* [Asteraceae] seeds; Rich, Gibson & Marsden, 1999), averaging several tens of plantlets. The limiting factor is the available material. Source populations are usually vulnerable as they are small and often have low reproductive success. *In vitro* culture might be an appropriate way to significantly multiply material, especially for plants that hardly reproduce by seeds or whose seeds are recalcitrants (Sugii & Lamoureux, 2004).

CHOICE OF THE SITES

Both enhancement and reintroduction programs involve transplanting new individuals in their site of origin. As the species had already developed on this site, it is *a priori* suitable for the restoration of the species. Unfortunately, such sites are often limited in size and in number, and therefore, restoration activities will often result in small, fragmented populations. Moreover, the environment has often changed between the population decline or extinction and the restoration. Thus, the success of the restoration is often uncertain (McEachern, Bowles & Pavlovic, 1994) and sites where the species has never grown before can be chosen instead.

The ecology of the selected site should correspond with the natural species habitat. In order to select a suitable site, the following criteria might be considered (Fiedler & Laven, 1996): (1) physical criteria: soil type, slope, altitude, flooding, trampling, or fires; and (2) biological criteria: dispersal vectors (pollinators and seed dispersers) and mutualistic associates (*e.g.,* families *Ericaceae* and *Orchidaceae* require mycorrhizes). If necessary, habitat corridors should be managed to connect restoration sites and allow natural dispersal. Predators and herbivores should be removed or controlled (Kirchner *et al.,* 2003).

Within the sites, plots are generally several tens of m^2. They are mostly fenced in order to protect them from large mammals. For trees or shrubs, plots are much larger and protective wire can be placed around each seedling.

Arenaria grandiflora example

DESCRIPTION OF THE NATURAL POPULATIONS

Arenaria grandiflora is a mountain Caryophyllaceae that mostly grows on cliffs and calcareous rocks of the south and central mountains of Europe. In France, it is frequent in the mountainous regions and very rare in lowlands (Tutin *et al.,* 1980). Two plain locations are extant: one is in the Parisian region (50 km south of Paris in the Fontainebleau forest), consisting of only 6 individuals in 2006; the other is situated 200 km southwest of Paris in the Loire valley and contains a few hundred individuals. The Parisian *Arenaria grandiflora* populations were firstly described in 1698. Since then, this species has been regularly reported in botanical publications. Around 1950 there were tens of individuals distributed among several populations (J. Pitton de Tournefort, unpubl. data). However, due to changes in forest management (*e.g.,* fire prevention, decrease of wood exploitation) and the abusive harvest of *Arenaria grandiflora* by naturalists and collectors of rare plants, many of the populations have been disappearing over recent decades. In 1995, for example, there were still about 40 individuals in 2 remnant populations: Mont Merle (MM) and Mont Chauvet (MC). However, the latter one (MC) went extinct in 2003 (Figure 1). The plain populations have been officially protected since 1991.

PRELIMINARY STUDIES

Preliminary studies were undertaken in order to determine the restoration plan. These studies were built on (1) observation of the individuals in the natural populations, (2) in-garden experiments, and (3) genetic studies with isozymes (Machon, Hunault & Moret, 1999; Machon *et al.,* 2001a).

ECOLOGICAL NEEDS

In the Fontainebleau forest, *Arenaria grandiflora* is always found on calcareous sands on steep slopes facing south, in open areas where competition is very low. The biotopes of plain populations (near the Loire) look very similar to those of Fontainebleau forest, while the mountain populations grow on cliffs or screes, suggesting that plants from plains and from mountains are different ecotypes of the same species.

BREEDING SYSTEM

The results of the genetic study with isozymes showed a high observed heterozygosity (Machon, Hunault & Moret, 1999; Machon *et al.,* 2001a). This suggests that the mating system of *A. grandiflora* is predominantly outcrossing, although flower bagging showed that selfing is possible (Machon, Hunault & Moret, 1999; Machon *et al.,* 2001a).

Arenaria grandiflora may reproduce vegetatively, since spontaneous cuttings are sometimes observed in the wild. For example, when old plants die, they often give rise to new plantlets through vegetative reproduction.

DEMOGRAPHIC DATA

The *Arenaria grandiflora* life cycle was determined by observation of plants in the natural populations (Figure 2).

Since 1995, we have followed the last 2 populations (only 1 remaining after 2003) of the Fontainebleau forest (Figure 1). Given the small number of remnant individuals, no statistical analysis was performed. Therefore, the results presented here are only qualitative tendencies. The majority of the plants did not flower. The few capsules that were produced contained mostly aborted seeds. Moreover, none of the very rare plantlets obtained from seeds survived, suggesting that the Parisian populations were too small to be viable. Furthermore, because of the low tolerance of this species to heat and drought, many of the plants died during the summers between 1995 and 1997 (Figure 1).

The plants in the Loire population were more vigorous, and no population extinctions have been recently reported.

GENETIC STUDIES

Increasing evidence indicates that *Arenaria grandiflora*'s populations from the Parisian region have been suffering from genetic alterations. In 1997, isozyme analysis showed low genetic diversity in the plain populations in comparison with the genetic diversity of mountain ones (Machon, Hunault & Moret, 1999; Machon *et al.,* 2001a). The 2 plain populations were, however, significantly differentiated from each other since the 2 systems (PGI and PGM) present exclusive alleles. Cultivation in controlled conditions (garden or greenhouse) showed the lack of vigour of the Parisian plants. Experimental crosses performed on clones of Parisian individuals showed a positive correlation between seed production and the geographic distance between the Fontainebleau forest and the source population of plants that provided pollen (Machon, Hunault & Moret, 1999; Machon *et al.,* 2001a). All the experiments that we carried out suggested that *Arenaria grandiflora* populations from Paris suffer from inbreeding depression and/or fixation of deleterious alleles by drift.

CHOICE OF THE RE-ESTABLISHMENT TRIAL

Our goal was to preserve *Arenaria grandiflora* in the forest near Paris. For that purpose, we proposed diverse types of plans: (1) *ex situ* conservation, propagating the remaining individuals in garden or by *in vitro* cultivation; (2) *in situ* management to maintain the biotope of this species and to protect it from competition with other species; and (3) re-establishment, as the first 2 plans were not effective by themselves.

PLANT MATERIAL

Given that the Loire and Parisian populations of *Arenaria grandiflora* were genetically differentiated using isozymes, but were located in a similar ecological environment, we opted for a re-establishment experiment mixing plants from the 2 origins. Since this was a pre-trial, the experiment was undertaken at sites in the Fontainebleau forest where the species had never been described and far (10 km) from the remnant population.

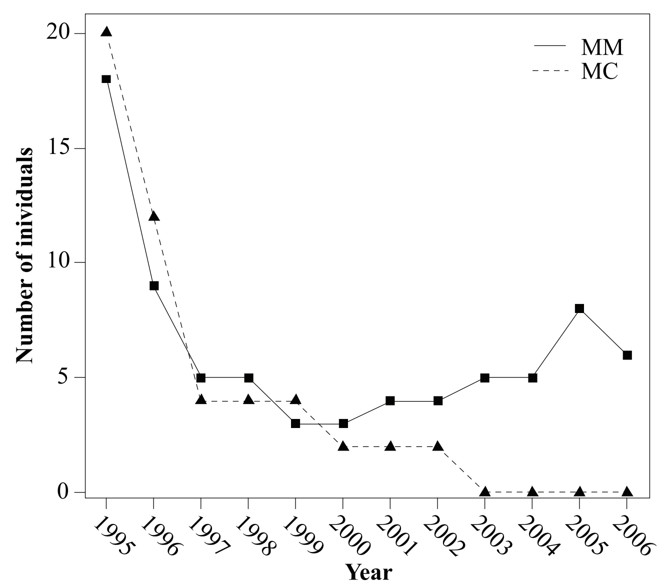

FIGURE 1. Number of individuals per natural population of *Arenaria grandiflora* in the Parisian region (Fontainebleau forest) from 1995 to 2006. The sites are Mont Merle (MM) and Mont Chauvet (MC).

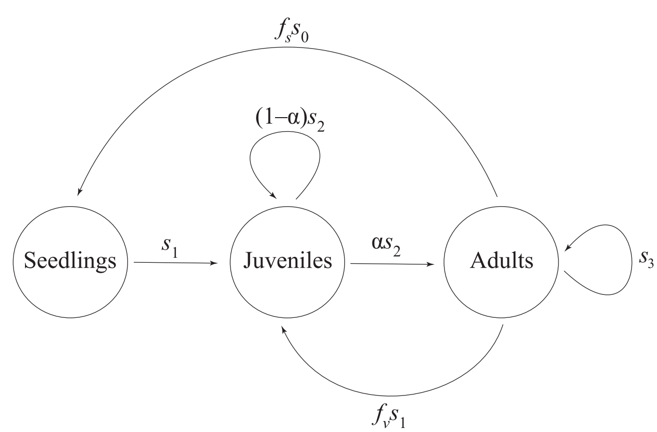

FIGURE 2. Life cycle of *Arenaria grandiflora*. s_0, s_1, s_2, s_3: survival probabilities between stages; α: flowering probability; f_s: fecundity or number of plantlets emerged per flowering plant; f_v: proportion of adults producing juveniles by vegetative reproduction.

The plants from Fontainebleau produced so few seeds that it was impossible to use them for the creation of new populations. Moreover, the plantlets phase seemed to be a very vulnerable stage. Thus, we chose to multiply plants by *in vitro* culture and to transplant adult plants in the wild. Cuttings were taken from wild plants at the plain localities (11 from the Loire valley, 6 from the Parisian population). During 2 y of multiplication via *in vitro* culture more than 1350 adult plants were produced. The repetitions of each clone were divided equally into 6 lots. Hence, 6 identical populations were produced (225 plants: 70 plants from the Loire valley and 155 plants from the Parisian region). Adult plants were ready to be transplanted in autumn 1999 (*i.e.,* after summer, the critical season for *A. grandiflora*). The number of individuals to be transplanted was similar to the size of the Loire populations. Mixing the plants from the 2 different origins resulted in high genetic diversity.

Restoration sites

The 6 created populations were introduced into the Fontainebleau forest in 3 sites (2 populations per site) considered ecologically suitable for *Arenaria grandiflora* (*i.e.*, calcareous sand soil and a south-facing slope) and located 10 km from the remnant natural population. The sites are called "Bois Rond" (BR), "Cuvier-Chatillon" (CC), and "Queue de Vache" (QV). Each of the 6 populations was transplanted in a 100-m^2 enclosure. The location of the plants within the enclosures was randomly chosen. The populations were precisely mapped using a grid of one m^2. The plants were numbered and labelled.

Follow-up to the experiment

The populations have been individually monitored since 1999. Every year, the surviving individuals are recorded and their size, number of flowers, number and size of capsules, and seed weight are measured. The population sizes have varied across all 3 sites since 1999 (Figure 3). Population sizes decreased in 2 sites. In the Bois Rond site, predation by rabbits destroyed a large proportion of the plants and the soil was not as calcareous as supposed initially. In the Cuvier-Chatillon site, competition with Poaceae reduced the development of the *Arenaria* populations. Invasion of the populations by *Brachypodium pinnatum* and *Brachypodium sylvaticum* has been favoured by the presence of the enclosures, which prevent grazing by wild mammals. In contrast, the population size of the Queue de Vache site tripled between 1999 and 2004, suggesting that reinforcement of the last natural populations is possible and that mixing populations might be successful. The more recent decrease in size of this population, however, remains unexplained (Figure 3).

Overall, our results suggest that plants from a distant (Loire) origin were at least as well adapted to their new environment as the plants of local origin (Parisian region). Fitness estimates indicate that plants from the Loire performed better than Parisian plants.

Current status and future goals

Although we are not yet able to give advice on the optimal composition of the created *A. grandiflora* population,

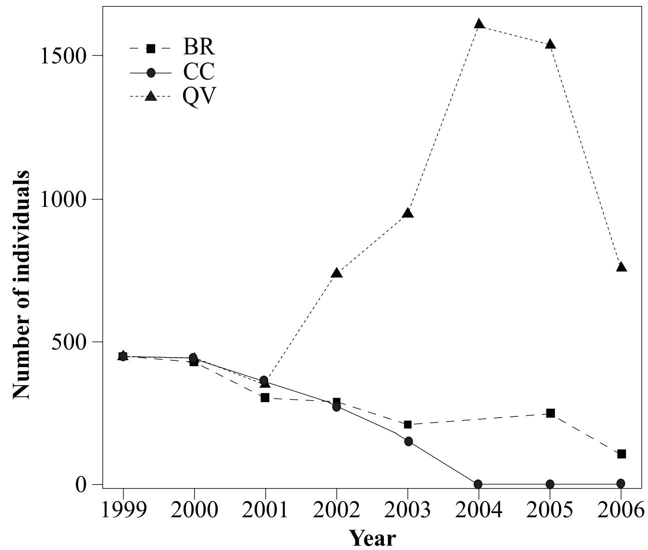

Figure 3. Number of individuals per artificial population of *Arenaria grandiflora* created in the Parisian region (Fontainebleau forest) from 1999 to 2006. The sites are Bois-Rond (BR), Cuvier-Chatillon (CC), and Queue de Vache (QV).

7 y of monitoring transplanted individuals has provided remarkable insights into restoration practices. The next step will be to assess the reproductive success of the plants from each origin and the genetic composition of different *A. grandiflora* cohorts using microsatellite markers. Such information will allow us to determine future management plans for the natural populations.

Conclusion

The aim of plant restoration programs is to create populations that are self-sustainable in the long term, although it is acknowledged that this may not be a one-step process. This review provides useful guidelines for such restoration plans. The previous failures in restoration programs were mainly due to unsuitable restoration sites or the low number of plants reintroduced in the wild. Populations below minimum population size are unable to persist, especially when genetic diversity is not enhanced at the foundation of the populations. Regular and thorough monitoring and managing of the restoration sites is also essential. Further theoretical and experimental studies will improve our understanding and management of successful plant species restoration.

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