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Complex *ex situ* - *in situ* approach for conservation of endangered plant species and its application to *Iris atrofusca* of the Northern Negev

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Abstract

We introduce a novel approach for conservation of endangered plant species in which *ex situ* collections maintained in natural or semi-natural environment are a part of a complementary *ex situ* – *in situ* conservation strategy. We provide detailed guidelines for 1) representative sampling of the populations; 2) collection maintenance; and 3) utilization for *in situ* actions. Our approach is the first that explicitly takes into account ecologically significant (i.e. adaptive) variation of plants in both *ex situ* and *in situ* conservation actions. We propose that an important part of the conservation strategy is preserving both neutral and adaptive genetic diversity through a quasi *in situ* conservation approach. Finally, we demonstrate this approach using a critically endangered plant species, *Iris atrofusca* from the northern Negev, Israel.

Keywords

In situ, *ex situ*, conservation strategy, relocation, translocation, local adaptation

Introduction

A large body of theoretical and empirical work has been devoted to particular questions about optimal conservation, such as the importance of inbreeding depression (Hedrick and Kalinowski 2000, Keller and Waller 2002), population size (Barrett and Kohn

1991, Ellstrand and Elam 1993), isolation (Newman and Tallmon 2001), genetic diversity (Lande and Barrowclough 1987; Newman and Pilson 1997) and outbreeding depression (Hufford and Mazer 2003, Tallmon et al. 2004). But, to-date there were limited attempts to conceptually unite different aspects of population viability as part of a conservation methodology. Such unification is especially lacking for *ex situ* conservation. In this paper we review the state-of-art in *ex situ* conservation with an emphasis on its utilization within a more general strategy having a final *in situ* output. Then we introduce a detailed approach for conservation of endangered species that integrates *ex situ* and *in situ* conservation as complementary and that could be used as a tool for finding an efficient solution to a particular conservation task. Finally, we present a study where this approach is applied for an endangered plant species.

Ex situ conservation

Ex situ conservation methods samples genetic diversity of species using certain criteria and store/propagate the collected material outside the natural environments in which the species grows (Heywood and Iriondo 2003). Importance of *ex situ* collections for conservation in situ was realized when collections in botanical gardens and arboreta helped implementation of population management and recreation (Falk 1987; Given 1987; Millar and Libby 1991). At the same time, limitations of their usefulness became evident. The latter include poor genetic or demographic management almost inevitably resulting in genetic erosion, artificial selection and spontaneous hybridization. To prevent/reduce negative effects of genetic drift, inbreeding depression and mutational meltdown, that all happen as a result of small (effective) population size of a collection, sampled individuals must be maintained separately or through controlled breeding and pedigree design. This introduces other limitations of *ex situ* collections, such as space limitations and high cost of maintenance.

A need of a conceptually sound link between conservation-oriented ecological and genetic research, and its routine application to *ex situ* management has been recognized (Maunder et al. 2004a). *Ex situ* conservation needs biologically effective, financially realistic and easy-to-use guidelines that can be applied to a wide range of situations. The development of such guidelines must take into consideration basic issues of conservation biology. Traditionally, the germplasm sampled for *ex situ* collection is supposed to represent potential adaptive variation within a species (Brown and Briggs 1991, Brown and Marshall 1995, von Bothmer and Selberg 1995). In case of limited resources for collecting and maintaining plants, minimal sampling can precede additional sampling, which will be performed upon availability of more resources (Brown and Briggs 1991). The key issue is choosing a limited, but representative, number of populations, using the correct criterion. This criterion, in our view, should be ecologically significant (i.e. not the potentially adaptive, but the currently adaptive) variation. Therefore, research that allows detection of spatial pattern of morphological, life history and fitness traits, should be the first priority tool for

providing sampling guidelines (Husband and Campbell 2004). Although variation revealed with molecular markers can provide valuable insights into the importance of different non-selective processes in species evolution, this information is secondary for making conservation decisions.

An endangered species is usually represented by small and isolated populations that already underwent strong effects of genetic drift and/or inbreeding, i.e. comprise a limited number of genetically different individuals (Aguilar et al. 2008). Open pollination in a sample from such a population may result in inbreeding depression due to high probability of mating between genetically identical or closely related genotypes. On the other hand, interbreeding of individuals from environmentally dissimilar habitats planted in close proximity often leads to outbreeding depression. These two risks rarely apply to obligate or predominant selfers, but can be extremely important for outcrossing, and especially for self-incompatible species. Outbreeding depression is an opposite, as compared with beneficial gene flow between genetically differentiated (isolated) populations, hybridization process. The parents do not necessarily have to be taxonomically distinct, viz. be recognized as different subspecies. Therefore, for an endangered species, creation of *ex situ* collections and decisions about suitable material for relocation/reintroduction should take into account the potential risks of inbreeding/outbreeding depression, in addition to local adaptation and spatial structuring of adaptive variation.

The above issues should be considered as the basic principles in developing an *ex situ* conservation approach that would be an integral part of a more general strategy with an ultimate final *in situ* output. Several approaches combining *ex-* and *in situ* conservation were proposed in the past, but none is satisfying as a general conservation strategy. For instance, the *inter-situ* approach proposes an off-site collection maintained within the natural habitat. This approach was considered potentially promising, but was not tested and no detailed methodology for practical use was developed (Husband and Campbell 2004). A slightly different approach are the "forest gene banks" (Uma Shaanker and Ganeshaiah 1997, Uma Shaanker et al. 2001, 2002). In this concept, a particular existing population acts as an *in situ* sink, into which genetic material from several source sites is introduced and maintained. Thus the genetically diverse sink population serves as a repository of the species gene pool and at the same time allows for random interbreeding. This approach might be useful in certain cases (lack of local adaptation, low genetic diversity, self-incompatability), but may lead to outbreeding depression when locally adapted genotypes are brought together. Therefore this concept cannot be used in a general application.

"*Quasi in situ*" conservation

Here, we propose the use of *ex situ* collections in natural or semi-natural environments as a part of a complex *ex situ – in situ* conservation strategy. Here below we provide detailed guidelines for 1) representative sampling; 2) collection maintenance; and 3) utilization for *in situ* conservation actions. The novelty of our approach is in that it ex-

plicitly takes into account potential local adaptation of plants in both *ex situ* and *in situ* conservation actions. An integral part of this strategy is preserving the species genetic diversity (both neutral and adaptive) through "*quasi in situ*" conservation.

The proposed strategy starts with an analysis of the species distribution to identify potential locally adapted populations or population groups. This analysis is crucial for understanding the extent of local adaptation and its spatial pattern. As intensity of local selection varies, either gradually with increase in distance or abruptly with change in a habitat, only knowledge of the local selection regimes can tell us whether material is adapted or not. Two main procedures exist to identify local adaptation: transplantation experiments (e.g. Joshi et al. 2001, Volis et al. 2002), and tests for outbreeding depression, when a locally adapted genotype is crossed with plants originating from elsewhere (Hufford and Mazer 2003). If local adaptation is important, the introduced genotypes must fit into the local biotic and abiotic conditions, i.e., it should come from within the area defined as that of intensive local selection, or from a habitat with closely similar local selection effects.

Experimental determination of a scale of local adaptation (Fig. 1) is a highly desired second step of the procedure. A potential for local adaptation and its spatial scale can be roughly predicted from knowledge of a species' breeding structure and life history. Self-pollination and short seed or pollen dispersal distance are known to be associated with a smaller scale of local adaptation than outcrossing and long-distance seed or pollen dispersal (Linhart and Grant 1996). However, these considerations are too general to

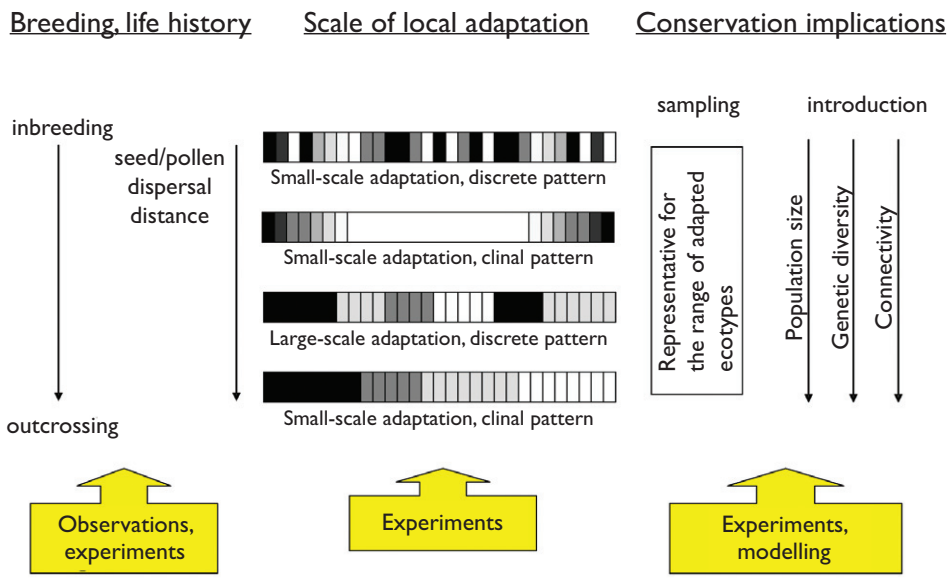

Figure 1. A scheme of relationship between (i) species properties (breeding structure, life history) and (ii) scale of local adaptation, and implications of (i) and (ii) for sampling and introduction. The scheme shows that predominant outcrossing and large dispersal distance are associated with large scale local adaptation, necessitating increased size, genetic diversity and connectivity of introduced populations.

be used as guidelines for conservation of a particular species because of numerous other factors, such as patterns of environmental variation (e.g. discrete or clinal), number of available habitats, a species' evolutionary potential, time since the last colonization, etc. Therefore, an experimental assessment of the scale of local adaptation is crucial. Such a test should include all habitats where the species is found (discrete variation) or locations along an environmental gradient (clinal variation). Of course, logistical considerations may limit a range of habitats or locations to be tested, or prevent testing for local adaptation at all. In this case variability in morphology, phenology and life history traits across a species' range must be known. This variability matching important environmental parameters (e.g. temperature, soil type, rainfall) or being associated with distinct habitats or vegetation communities are indications of local adaptation.

If phenotypic or genetic variation is spatially structured, even though it is not a result of natural selection, it may represent distinct evolutionary lineages within species, being an important part of the species' diversity. This variation, although neutral, is important for preserving species evolutionary potential, i.e. ability to adapt to future climate or habitat changes. As neutral genetic variation in many cases is not reflected in phenotypic or in molecular markers variation, its existence must be presumed when a species consists of populations not connected through gene flow.

With knowledge about spatially structured adaptive and neutral variation, a sampling design is worked out. The optimal design for *ex situ* collection is a stratified one, with a lower level (of neutral variation) nested within a higher one (of adaptive variation). Practically, this means that for a species present in several habitats (e.g. soil types, regions of different aridity, vegetation communities), sampling in each habitat must include several geographically isolated populations. A representative number of spatially separated individuals must be collected in each population, to ensure sampling of different genotypes.

The next step is planting the germplasm sampled as living collection. In the *quasi in situ* approach, a choice of a site must take into account local adaptation (tested or presumed). This means that there must be a close environmental match of *ex situ* location with locations of sampled natural populations. A close match can be biologically meaningful only if the *ex situ* location is in a natural (or at least semi-natural) environment. In addition, this site should be protected by national law and practically (regularly) inspected by rangers. In our view, different classes of strictly protected territories are the areas that fully satisfy these requirements. An optimal design would be planting several populations representing a particular eco-geographical region or habitat in a protected area in the same eco-geographical region or habitat. A representative number of genetically different individuals per population should be planted separately at distances, allowing subsequent identification of planted genotypes. This is important for both estimating rate of survival and enabling controlled pollination, if necessary. Following the recommendations of the Center for Plant Conservation in the United States (Maunder et al. 2004a), number of plants per population should be 10 to 50, and five populations per habitat or eco-region should be sampled.

A comparison between *quasi in situ* and traditional *ex situ* conservation in botanical gardens is summarized in Table 1. We argue that the *quasi in situ* approach provides

better representation of genetic diversity, increases chances of germplasm survival, and better suits the purpose of propagating material for *in situ* conservation actions.

In situ conservation aims at either enhancement of existing populations or creation of self-supported new populations via reintroductions and translocations, using sampled or propagated material (reviewed in Bottin et al. 2007, Menges 2008). When a natural population exists, or existed in the recent past, a choice of material is quite straightforward. A large population from the same or the geographically closest population within the same habitat would be the best choice. If, however, relocation is planned, viz. introduction of material into locations where a population never existed, a decision is more problematic. There are many examples of unsuccessful relocation into sites that seemed highly suitable and were located in close proximity to a location where natural populations had been extirpated (e.g. Holland 1980, Morton 1982, Cranston and Valentine 1983). This implies that the recommendation by Schaal and Leverich (2004) to use for relocation a large sample from the closest population representing the same habitat, should be treated with caution and only applied when data on a species' environmental requirements are very limited. A much better option is a limited relocation within an experimental framework, and a full relocation in those sites where survival and reproduction are high.

As soon as the relocation sites are chosen, material for propagation may be taken from natural or *ex situ* collections. The major issues are required origin, genetic diversity and quantity. Acquiring sufficient quantity of propagation material (seeds, bulbs, root cuttings, saplings) from the closest natural population can negatively affect the latter's growth rate and threaten its viability. In addition, single source material may lead to inbreeding depression and high susceptibility to diseases. *Quasi in situ* collection may effectively solve both problems. Plants, grown in the collection, are (presumably) locally adapted and genetically different as they originate from several populations. Additionally, naturally occurring cross-pollination of plants in a collection should neither lead to breakdown of co-adapted gene complexes, nor to dilution of local adaptation because all plants in the collection originated from the same environment and no maladapted genes will participate in recombination and segregation. Therefore, the offspring of cross-pollination in a collection should well suit the relocation purpose, and can be collected in large quantities to meet the needs for successful relocation.

The last step in the *quasi in situ* strategy is determination of spatial parameters of introduced populations (size, distance from the nearest population) and monitoring of relocation success. Again, as with choosing a relocation site, experimentation should

Table 1. A comparison between *quasi in situ* and *ex situ* conservation.

Parameter	Ex situ	Quasi in situ
Space for maintaining the collection	Limited	Less limited
Suitability of environment	Usually un-suitable	Suitable
Maintenance and renewal of material	Artificial	Natural
Cost	High	Very low and only at the initial stage

be a common practice when several populations of different size are planted and monitored over a number of years.

Application: *Iris atrofusca* of the northern Negev as a case study

Iris atrofusca Baker (Fig. 2) belongs to the section *Oncocyclus* (Siems.) Baker (Iridaceae) that are characterized by dense clonal growth and conspicuous large, mostly dark flowers that grow individually on a stem (Avishai and Zohary 1980, Sapir et al. 2002). Eight species of *Oncocyclus* that grow in Israel (Feinbrun-Dothan 1986, Danin 2004) have high conservation priority (Sapir et al. 2003) and are included in the Red Data Book of the country (Shmida and Pollak 2007). *Iris atrofusca* is one of the most threatened species of *Oncocyclus* irises in Israel these days (Shmida and Pollak 2007).

Iris atrofusca is relatively widely distributed. It occurs from the northern Negev in the south to the Golan heights in the north. This distribution is the widest of all *Oncocyclus* species in Israel. Identification is not always easy. Sapir et al. (2002) showed that morphologically it does not differ from its closest relatives, *I. haynei* and *I. petrana*. Morphological traits are also associated with the aridity gradient (Arafeh et al. 2002, Sapir et al. 2002). However, morphological and genetic analyses indicated that *I. atrofusca*

Figure 2. Leaf fans, flowers and rhizomes of *Iris atrofusca* from the Goral Hills, northern Negev (watercolor by Irene Blecher © 2006).

populations of the northern Negev form a cluster within the general pattern (Arafeh et al. 2002, Sapir et al. 2002), and might even represent a separate taxon (Kushnir 1949).

The habitats of *I. atrofusca* in the northern Negev are the most vulnerable throughout its distribution. In the last decade, *I. atrofusca* populations of the northern Negev have been suffering mainly from anthropogenic disturbance that decreased population sizes, with some populations becoming extinct. These disturbances include urbanization, infrastructure works, intensive and extensive agriculture, overgrazing, forestry works, and illegal Bedouin settlements. Recently, a plan for expanding the area of Beer Sheva, the main town of the northern Negev, is threatening the largest and the densest populations of *I. atrofusca*, which grow in Goral Hills, north of the town. These issues lead to urgent research of *I. atrofusca* populations in the Negev. Here we present studies we did under the guidelines we drew above for the *quasi in situ* conservation approach.

Methods

Research area

Iris atrofusca grows in the northern Negev in two main groups of populations: in Goral Hills (central coordinates: 31°18'N 34°48'E), north to Beer Sheva, and Arad Valley (central coordinates: 31°16'N 35°07'E) (Shimshi 1979/8).

The two regions differ in climate. While the Goral Hills area is above the 200 mm isohyet (semi-arid conditions), the Arad Valley is close or below to the 150 mm isohyet, which indicates arid conditions (Atlas of Israel 1985, Jaffe 1988).

The topography of the Goral Hills area is mostly slopes of shallow hills. The angles of the slopes are up to 20%. The soil is a shallow calcareous lithosol overlying fractured Eocene limestone (Shimshi 1979/80). Depressions between the hills are filled with shallow loessial soil. Arad Valley, on the other hand, is a relatively flat plain (with wadis and gullies), covered with Quaternary aeolian loess of considerable thickness (> 2 m), with some isolated outcrops of calcareous lithosols (Shimshi 1979/80), which are mostly the heads of insulated hills.

In hard and fissured limestone and dolomite with calcareous lithosol some of the rain water penetrates the soil and is accumulated in the fissures and crevices, where it is protected from direct evaporation. Loessial soils have a different moisture regime. Due to the high moisture holding capacity of the fine-grained substratum, some of therain water is absorbed by the upper soil layers, but most of this water is consequently lost by direct evaporation from the soil surface (Danin 1988).

Inventory and demographic observations

A field survey based on previous knowledge on the distribution of the species in the northern Negev was conducted in 2006. The survey aimed at documenting the pre-

cise locations of populations, the distributing area of each population, and to record ecological conditions and anthropogenic impacts. In each population, clumps of *Iris atrofusca* were recorded for their size, estimated by the diameter of the clump.

To assess population growth rates, we started, in 2006, a detailed census of two populations representative of two regions, the Goral Hills and Arad Valley (Gvaot Goral, G-G, and Tel Arad, T-A, respectively) (Fig. 3). Two permanent observation plots were established: 120 m x 6 m (G-G) and 60 m x 6 m (T-A), respectively). In March 2006 we counted all individuals within the plots and classified them as either immatures (1st or 2nd year juveniles with a single fan), vegetative (non-flowering, but with > 1 fan) adults or reproductive adults (Fig. 4). We marked each established clump (=genet) of *I. atrofusca* individually, measured its diameter and counted the number of leaf-fans (=ramets). Also in April 2007 and 2008, we recorded number, size, and reproductive status of adults in the plots. Each season (2006, 2007 and 2008) we calculated the average number of fruits per reproducing plant (i.e. a clump comprising >1 ramets), average number of seeds per fruit, and resulting fecundity. Measurements and counting were done when plants started to senesce.

Since the actual age of individual plants of *I. atrofusca* in the field can not be determined, the population structure analysis was based on the number of individuals

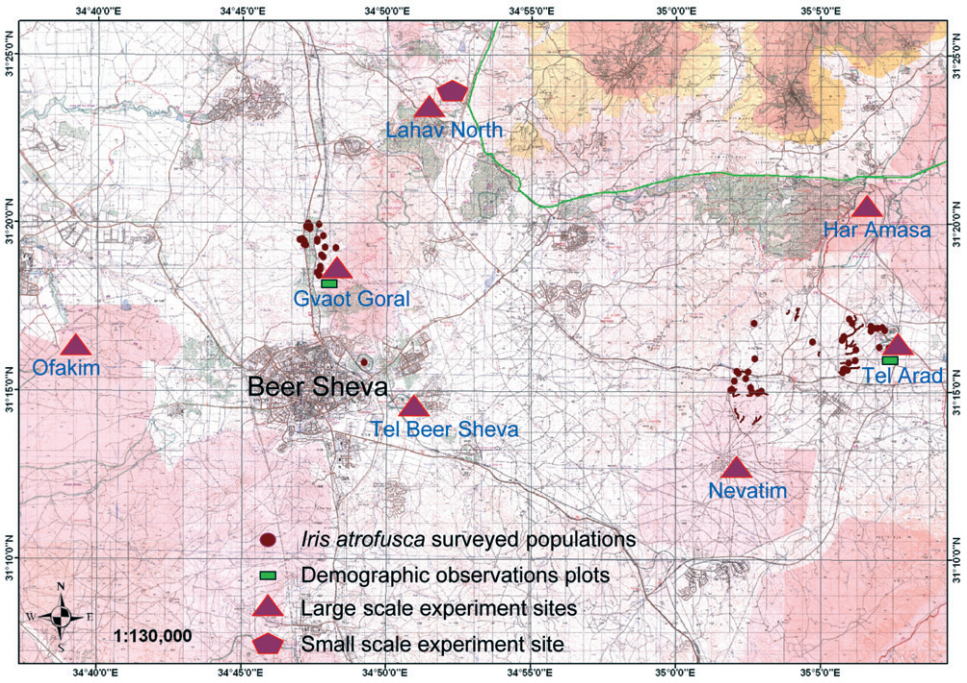

Figure 3. Map of populations surveyed, experimental relocation sites and populations in which permanent demographic observations plots were established.

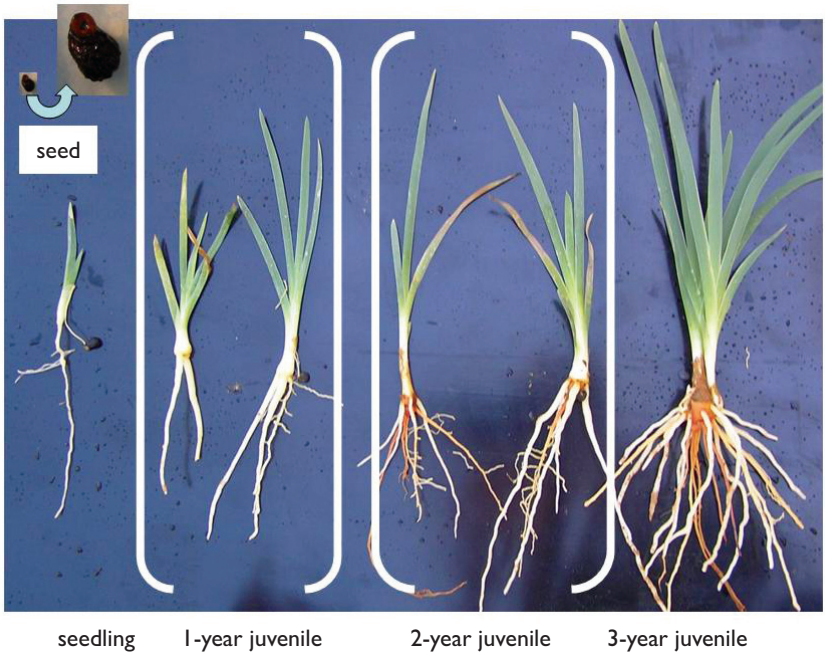

Figure 4. Plants of *I. atrofusca* followed from seed germination on and representing different age-stages (seed, seedling, juvenile and vegetative adult).

in the different ontogenetic stages of the life cycle. During the years 2005 to 2008 we followed the germinated seeds to distinguish age-stages in *I. atrofusca*. We identified the following age-stage categories (Fig. 4):

1. Seeds;
2. Seedlings (individuals developed shortly after germination of seeds, with cotyledons and often with one pair of leaves);
3. Juveniles (individuals with a single leaf fan comprising more than two leaves and having a poorly developed root system); a major difference between 1-year and 2-year juveniles is in the development of the root system, where the latter starts to develop a rhizome;
4. Vegetative adults or immatures (non-flowering individuals with more than two leaf fans and fully developed root system, which has a developed rhizome);
5. Generative adults (individuals bearing flowers).

Oncocyclus plants are dormant between the end of the flowering season (end of spring) and the start of the next winter. Our observations indicate that plants can stay dormant during not only summer, but also during the next growing season, from fall to spring (Volis & Blecher, pers. obs.). This adds another ontogenetic stage for adults – dormants, which will be verified as more demographic data become available.

Adult plants can be distinguished from juveniles in the field by the compact assemblage of leaf fans, which emerge close to each other from the below-ground rhizome (Fig. 4). All these leaf fans are genetically identical individuals (ramets) that may become independent plants after fragmentation of the mother rhizome (genet).

Plant sizes and number of fans per clump were log-transformed and analyzed across years and habitats using repeated-measures ANOVA. Only alive and non-dormant adults were analyzed for these two traits.

Soil seed-bank and germination trials

We created three experimental permanent soil seed banks and started monitoring the fate of the seeds in fall 2005 at Tel Arad National Park (Fig. 3). Seeds of *I. atrofusca* were collected by MB from plants in proximity to the plots under observation in 2005. The seeds were buried at three sites along the slope in: (1) plastic trays filled with soil of the site of transplanting and containing one spikelet per cell (221 seeds per tray, one tray per site), placed about 2 cm below ground level, and (2) in furrows (100 seeds per furrow, two furrows per site).

Similar soil seed banks were established in fall 2006 at Gvaot Goral site (Fig. 3). Two trays, each containing 221 locally collected seeds, were buried at the top and the bottom of the hill. The experimental soil seed banks were monitored for seed germination during 2006 to 2008.

Effect of rhizome initial size on growth and flowering

This experiment was conducted during the growing seasons of 2005 and 2006. We used only rhizomes with one distinct bud to test the effect of initial rhizome weight on probability of flowering. We also measured several morphological traits to identify potential morphological indicators for probability of flowering. Rhizomes were planted in 3-liter pots filled with loess soil, one weighted rhizome per pot. Pots were placed 25 cm apart in a nethouse, and watered regularly with 2-liter/hour drippers, one dripper in each pot. During the experiment (November – April) plants were getting natural rainfall (208 mm) plus supplementary watering (equivalent to 95 mm of rainfall), to compensate for the higher evaporation rates from the pots. At first sign of leaves senescence the following measures were taken: length and width of the longest leaf, diameter at the base of the leaf fan, number of ramets, and number of flowers. After complete drying out of above-ground biomass the rhizomes were dug out and weighted. The sample size was 204 plants. Large rhizomes rescued by MB in 2005 from the Goral Hills (road-building strip for new railroad tracks) that represented a group of (potentially) independent ramets were cut into pieces and used in this and the following experiments.

Creation of quasi in situ gene banks

Between 20 to 50 large genets of *I. atrofusca*, comprising many ramets, were sampled from four populations from Goral Hills and Arad Valley regions. Populations were chosen based on: (1) the threat of habitat destruction – the populations chosen were critically endangered (construction, agriculture, etc.) and required immediate relocation; and (2) their representation for the distributional range of *I. atrofusca* in the northern Negev. The plants were planted in two replicates at both Tel Beer Sheva and Tel Arad National Parks (Fig. 3) that represent ecological conditions like those of Goral Hills and Arad Valley areas, respectively. However, as the two population groups (Goral and Arad areas) were found to differ in habitat, demography and morphology (Shimshi 1979/80; Blecher 2007 and this study), we decided that Tel Beer Sheva and Tel Arad National Parks will harbor populations from their respective regions only, and the populations planted outside their region of origin will be relocated to the refuge in their respective region at the next stage of the project. Meanwhile, we are monitoring the transplanting success of plants of different origin across the two regions.

Relocation experiments

Rapid disappearance of *I. atrofusca* populations in the Negev necessitates measures of species conservation, such as relocation to safe areas, protected by law. In order to determine species' habitat preferences we set relocation experiments at two scales, large (tens of kilometers) and small (hundreds of meters), respectively, using rhizomes rescued from sites of habitat destruction and immediate threat for the plants, i.e. from populations that required relocation.

Large scale relocation experiment

Two sets of five large (> 20 g) and 15 small (5–10 g) rhizomes of *I. atrofusca* of Arad Valley and Goral Hills origin were planted in six locations that embraced the whole species range in the Negev and beyond it. The locations were: KKL experimental site near Ofakim, Tel Beer Sheva National Park, Nevatim Basis, Lahav North Nature Reserve, Tel Arad National Park, Har Amasa Nature Reserve (Fig. 3). In spring 2007 and 2008 we recorded the numbers of surviving plants, flowers and fruits per plant.

Small scale relocation experiment

Rhizomes rescued in spring 2006 in the Goral Hills area (construction of new railroad tracks) were planted in fall 2006 in sets of 62 rhizomes at 22 microhabitats in Lahav

North Reserve (Fig. 3, 9). Each set comprised the following size classes: <5 g (14), 5–10 g (10), 10–20 g (23), 20–30 g (10), 30–40 g (3) and >40 g (2). In spring 2007 and 2008 we recorded the numbers of surviving plants, flowers and fruits per plant.

Results

Distribution and habitats

The results of this survey (Table 2) clearly show that in the Arad Valley about a third of the plants of *I. atrofusca* grow in wadis. In the Goral Hills area, no population was found in wadis. Detailed geographical interpretation of data on *I. atrofusca* survey in the northern Negev, including categorization of the populations for protection purposes, is presented in Blecher (2007) with proposals for new protected areas and enlargement of the existent Parks.

The two populations studied (Gvaot Goral and Tel Arad) differed in average plant density, estimated in spring 2006 (0.88 vs. 0.30 plants/m^2 in G-G and T-A, respectively). There was a marginally significant difference between two populations in clump size, with clumps at G-G being consistently larger during three years than at T-A. The number of ramets per genet exhibited significant season/population interaction. This trait was more constant over the years at G-G than at T-A (Table 3).

The two populations differed in the stage structure, with juveniles comprising 86%, 70% and 46% vs. 23%, 24% and 20% of established plants (over three years) in G-G and T-A populations, respectively (Table 4). Percentage of flowering adults and average fruit-set per reproducing plant over three years were higher in G-G than in T-A population (40±13% vs. 67±10% and 1.61±0.38% vs. 0.52±0.30%, respectively). The two populations also dramatically differed in fecundity (Table 4).

Germination

Low germination rates were observed in the soil seed banks established in 2005 at Tel Arad National Park. During three seasons, 2005–6, 2006–7 and 2007–8, no germination event was recorded in any of the buried trays with seeds. In furrows, no germination was observed in 2005–6 and 2006–7, but in 2007–8 germination fraction was

Table 2. Number of plants and distribution of *Iris atrofusca* in two geographic regions.

	Total distributing area (Hectare)	**Total cover of plants* (m^2)**	**Total number of clumps**	**Hills and slopes**	**Wadis**
Goral Hills	19.3	186	1968	1968	0
Arad Valley	34.8	439	4716	2931	1785

* Total cover of plants is a summary of all clumps.

4%, 15% and 12% (hill top, middle and foot, respectively) In the Gvaot Goral site, where 2 trays (experimental soil seed banks) were buried in fall 2006, one seed germinated in the following winter (season 2007–2008). These results suggest strong seed innate dormancy in the first year after dispersal with increase in germination fraction in following years.

Table 3. Repeated measures ANOVA of the effects of population and season on clump size and number of ramets per genet (top) and means ± S.E for each season (bottom). G-G – Gvaot Goral population, T-A – Tel Arad population.

Source of variation	**DF**	**F**	**F**	
		Clump size	Ramets per genet	
Population	1	2.91†	2.38 ns	
Error	109			
Season	2	0.59 ns	1.82 ns	
Season * Population	2	0.91 ns	6.15**	
Error	218			
Season	Clump size (cm)		Ramets per genet	
	G-G	T-A	G-G	T-A
2005–6	24.0±2.6	19.8±2.4	21.5±3.3	18.6±3.2
2006–7	23.1±2.6	19.8±2.5	18.3±2.5	15.0±2.5
2007–8	25.1±2.5	22.6±2.8	18.0±2.3	21.8±2.8

** $p < 0.01$; † $p < 0.10$; ns not significant.

Table 4. Life table for two populations of *I. atrofusca* during the seasons 2005–6, 2006–7 and 2007–8. The table does not account for soil seed bank present at the start of observations. Numbers are for the whole plot.

Population/stage	**Season**		
	2005–6	**2006–7**	**2007–8**
Gvaot Goral (G-G)			
Seeds	–	3238	2318
Juveniles	357	142	90
Non-reproducing adults	17	16	70
Reproducing adults	43	46	35
Fecundity (seeds/repr. plant)	75.3	50.4	16.4
Tel Arad (T-A)			
Seeds	–	592	199
Juveniles	25	23	22
Non-reproducing adults	66	33	67
Reproducing adults	19	38	19
Fecundity (seeds/repr. plant)	31.2	5.25	8.4

– = not estimated

Effect of rhizome initial size on growth and flowering

The results of this experiment (Table 5) clearly show that sexual reproduction (i.e., production of a flower) in *I. atrofusca* depends on the rhizome weight and two size-related parameters, namely length of the leaves and base diameter. The minimal rhizome weight for flowering appears to be around 2.7 g-3.0 g, but probability of flowering for such plants is less than 10% (Fig. 5). The optimal rhizome weight with reasonably high probability of flowering (around 50%) is above 4 g (Fig. 5).

Creation of *quasi in situ* living collections

Two years after planting, survival rates in two living collections were equally high, approximating 100%. Percent of flowering plants was substantially higher in Tel Beer

Table 5. Results of multiple logistic regression testing effect of five predictor variables on probability of flowering.

Parameter	Wald Statistics	p
Intercept	0.24	0.6210
Rhizome weight	30.76	< 0.0001
Leaf length	13.46	0.0002
Leaf width	0.15	0.7008
Base diameter	4.20	0.0403
Number of leaf-fans	1.26	0.2618

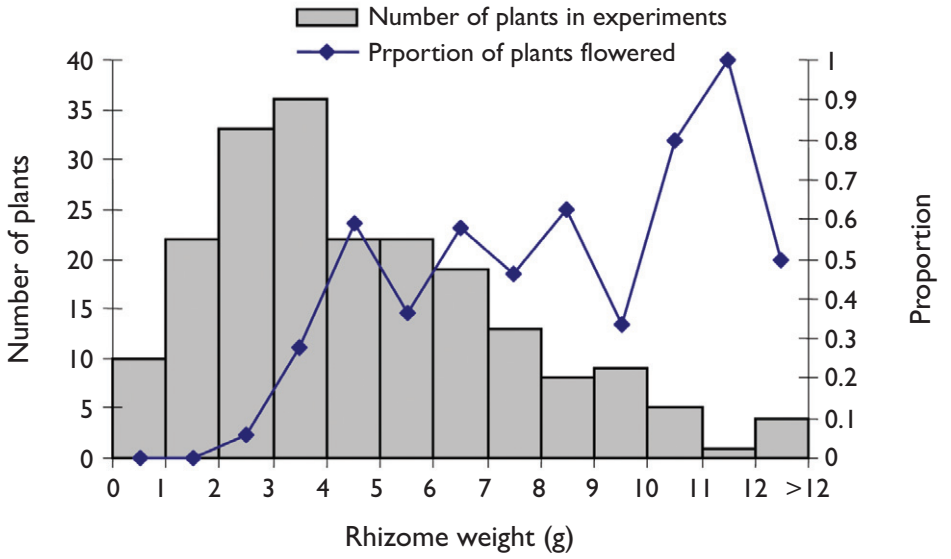

Figure 5. Proportion of plants flowering and total number of plants of different rhizome weight classes in the experiment described in the text.

Sheva National Park than Tel Arad National Park, while a difference in percentage of plants that produced mature fruits was less pronounced (Fig. 6). Plants of native geographic origin had no advantage at either location.

A high percentage of flowering plants at one location (Tel Beer Sheva National Park) indicates high potential seed productivity in the living collection. The low percentage of plants that set fruits appears to result from low numbers of pollinators in this area. Therefore the genetic refuges can be a source of seeds for relocation once artificial pollination is provided.

Large scale relocation experiment

High survival rates were observed in the first year after introduction at all locations, and the highest number of reproducing plants was observed at Ofakim and Lahav North (Fig. 7). At Har Amasa, plant above-ground biomass was browsed by grazing livestock, thus, assessment of reproduction was not possible. Two years after the introduction a difference in plant survival rates among the locations started to become obvious (Fig. 7). Grazing at Har Amasa again prevented assessment of plant reproduction.

The most unexpected and counterintuitive result was zero reproduction at two experimental sites established in close proximity to the natural populations, G-G and T-A. In both cases experimental locations were established on an adjacent hill slope. This indicates the importance of microscale conditions for relocation success. At the

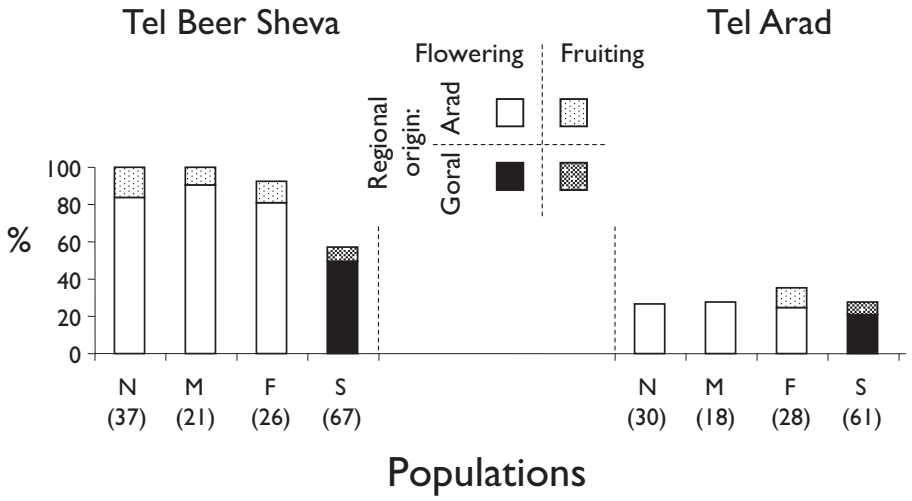

Figure 6. Population name, number of genetically distinct individuals (in parentheses), and percentage of flowered and fruited plants two years after creation of two *quasi in situ* collections in Tel Beer Sheva and Tel Arad National Parks, respectively.

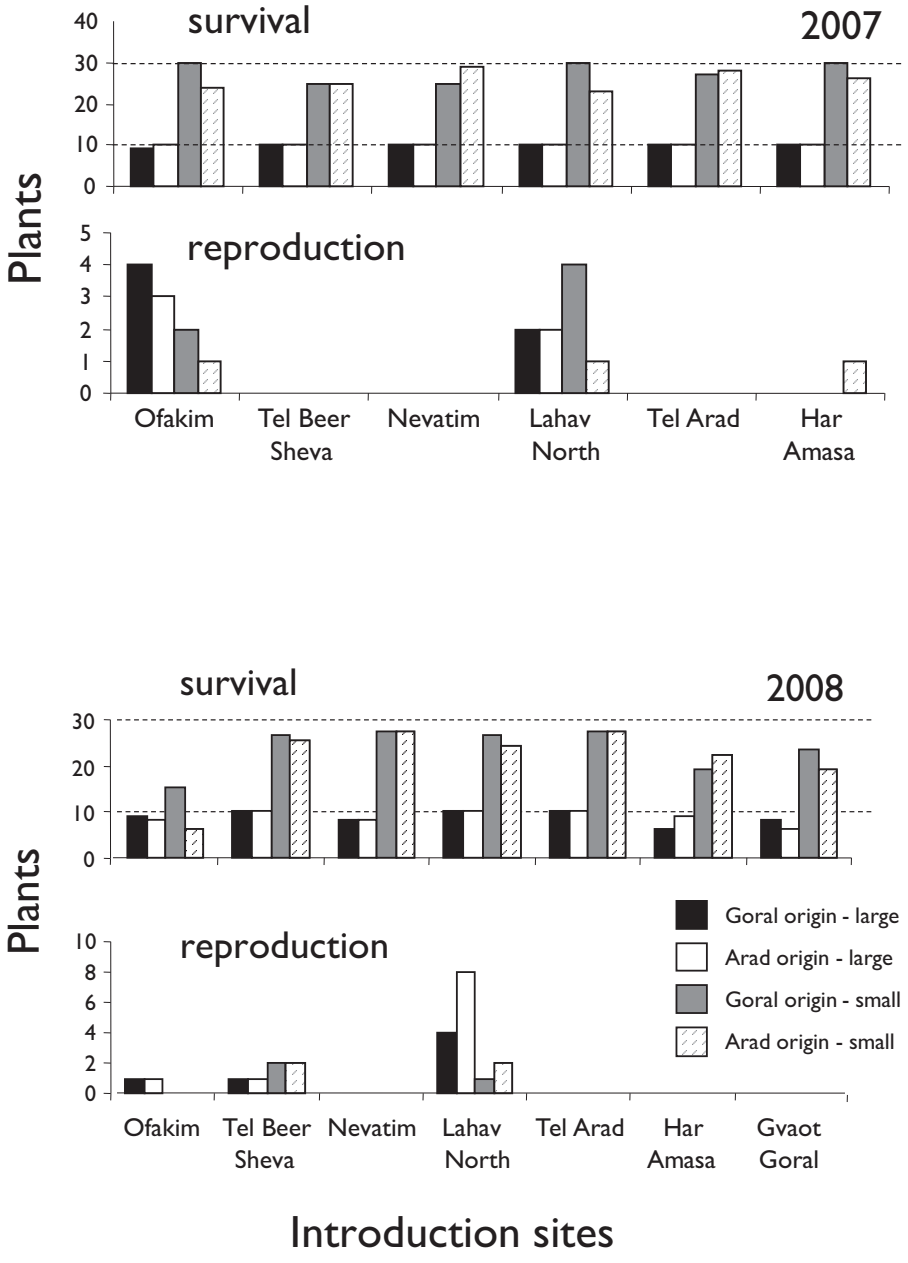

Figure 7. Survival and reproduction of *I. atrofusca* one year after experimental introduction. Ten large (> 20 g) and 30 small (5–10 g) rhizomes of Goral Hills and Arad Valley origin were introduced at each site.

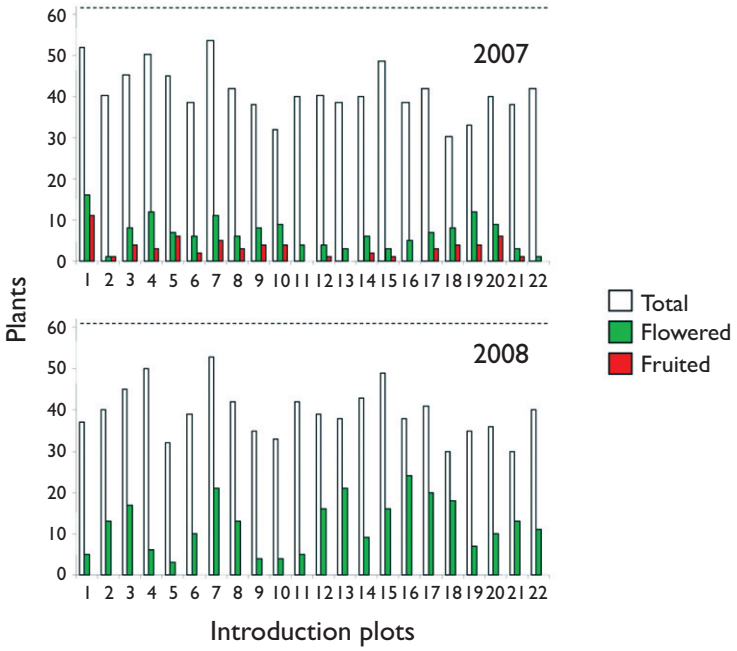

Figure 8. Survival and reproduction of *I. atrofusca* one year after experimental introduction at Lahav North Reserve. Sixty-two rhizomes of Goral Hills origin with equal representation of different size classes were introduced at each introduction plot.

Lahav North Reserve both survival and reproduction of plants were consistently high during two years of observations. This strongly supports our decision made in 2006 to start experimental microscale relocation at the Lahav North Reserve. At the Ofakim site reproduction was high in the first year but dropped dramatically in the second year after planting.

Small scale relocation experiment

The number of plants observed at the 22 microsites in the Lahav North Reserve one year after introduction ranged from 25 to 52 plants (out of 62 introduced) with no significant difference between microsites (G-test, $G_{.05,21} = 9.2$, $p > 0.05$; Fig. 8). The microsites did not differ either in the number of plants that set fruits (G-test, $G_{.05,21} = 32.4$, $p > 0.05$), but differed in numbers of flowering plants (G-test, $G_{.05,21} = 36.8$, $p < 0.05$).

Two years after the introduction, the range of surviving plants per microsite was between 33 and 53, generally higher than the previous year records. This indicates that some plants were not counted in the first year census, perhaps due to rhizomes dormancy. As in the first year, no microsite difference was observed for plant survival in the second year (G-test, $G_{.05,21} = 20.0$, $p > 0.05$), but numbers of reproducing plants were significantly different among microsites (G-test, $G_{.05,21} = 74.2$, $p < 0.001$; Fig. 8).

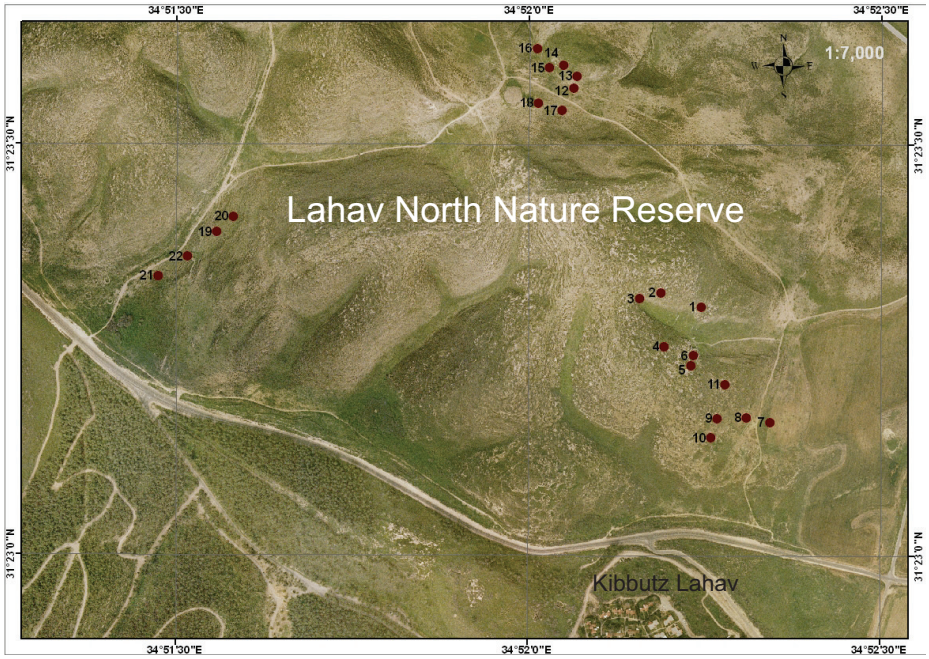

Figure 9. Map of the Lahav North Reserve with 22 microsites at which identical sets of *I. atrofusca* rhizomes were planted.

Contrary to the first year, no flower set fruit at any microsite in the second year. All the flowers were consumed by grasshoppers and caterpillars, indicating the important role of biotic interactions at the Lahav North site.

Discussion

Distribution in the Negev and population demography

The observed differences in stage structure (i.e. the frequency of life-cycle stages) between the two populations correspond to two types of demographic behavior, the "invasive" or "dynamic" (Gvaot Goral) and "normal" or "stable" (Tel Arad) (Rabotnov 1969, 1985, Oostermeijer et al. 1994). The former is characterized by a higher proportion of immature plants relative to the adults, while in the latter the adults predominate and the young individuals are low in number. These two population types are usually associated with different succession stages of the local vegetation community, but in the two *I. atrofusca* populations studied no difference was apparent with respect to the succession stage. One major difference between the two population locations is in aridity, and the observed difference in a proportion of immature plants appears to be due to higher survival of seedlings (although survival of juveniles does not differ) at less xeric Gvaot Goral site. Nonetheless, it is too early to

draw conclusions about the long-term dynamics of these two populations. The latter requires a multi-year census coupled with records of annual rainfall and assessment of grazing pressure.

The two groups are separated from each other by a distance of ca 20 km with hardly any gene flow between them. Different environmental conditions (soil, rainfall) and anthropogenic impact (intensive grazing vs agriculture) may have caused differential selective responses in the two regions. Therefore an *I. atrofusca* conservation strategy must be based on the assumption that ecologically important (i.e. adaptive and caused by biotic/abiotic environmental variation) exists within *I. atrofusca* in the Negev and regional criterion (Goral Hills vs Arad Valley range subdivision) is a first approximation of this variation. This assumption has several implications for this species *ex* and *in situ* conservation.

Ex situ implications

If plants in two regions are adapted to different environmental conditions, sampling and maintenance of living collections must be done for each region separately. Mixing or physical proximity of plants having different regional origin must be prevented. If, due to logistical limitations, plants are maintained in the same location, measures must be taken to prevent spontaneous hybridization (e.g. removal of immature fruits). On the other hand, interbreeding of plants originating from different populations within the same region, is desired to decrease risk of inbreeding depression and self-incompatibility. The latter negative effects were detected in fragmented populations of *I. bismarckiana*, a close relative to *I. atrofusca* (Segal et al. 2007).

In our study, plants from five populations of *I. atrofusca* in the Negev were planted at two National Parks, creating two duplicates of the same living collection. After careful study of region-specific environmental conditions, anthropogenic impacts and population demography we concluded that we should divide our collection based on a regional criterion. In spite of the initial proximal planting of plants from two regions, no spontaneous hybridization occurred during two years of collection maintenance because of the precautions described above.

After removal and re-planting of populations representing the Goral Hills area, into their region-specific location in Tel Beer Sheva National Park, and populations from the Arad Valley area into their region-specific location at Tel Arad National Park, the next step in applying *quasi in situ* conservation, using plants in the living collections for seed propagation. In a case of low fruit set due to limited availability of natural pollen vectors (*Eucera* bees; Sapir et al. 2005) randomly applied artificial pollination should be performed. The seeds obtained can be treated to reduce strong innate dormancy, and germinated in mass. Young plants with rhizomes acceding 4 g can be used for *in situ* actions, which should be performed with plants of proper regional origin.

In situ implications

Rapid destruction of *I. atrofusca* natural environment in the Northern Negev due to heavy anthropogenic impact on the one hand, and lack of nature reserves that contain populations of *I. atrofusca* in the Negev, on the other hand, leaves very limited options for conservation of this species. Declaration of new protected areas in the northern Negev is very problematic because of economic, demographic and political issues. There is virtually no vital alternative to relocation, i.e. introduction of the species into presumably suitable protected areas with no previous records of the species. At the same time, the choice of such areas for *I. atrofusca* in the Negev is limited.

It is too early to draw conclusions from our relocation experiments, started in 2006, about factors limiting species distribution. At least several more years are needed for reliable conclusions, because among-year fluctuations, as well as long-term effects should be considered. However, some general considerations about a choice of relocation material can be done even at this stage. Using regional subdivision as a guideline for successful relocation, creation of a new population within Goral Hills or Arad Valley region should be done using material from the same region. If, however, a new population location can not be ascribed to one of these regions, possible options include material of single regional origin (either Goral or Arad) or a mix of two. Although lack of local adaptation is not an issue here, hybridization of two ecotypes may result in a disruption of coadapted gene complexes, high genetic load and low average fitness of plants in the new population. As a result relocation success may be low. Without relocation experiments, it is impossible to decide which of two plant origins is more suitable.

Conclusions

We conclude that the proposed approach assessing ecological importance, and using this information for both, *ex* and *in situ* conservation is suitable for endangered species that are distributed over areas with complex and variable ecological conditions. We hope that detailed guidelines developed from the above approach for: (1) representative sampling of populations; (2) collection maintenance; and (3) utilization for *in situ* actions will be used as a tool for efficiently solving specific conservation problems.

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