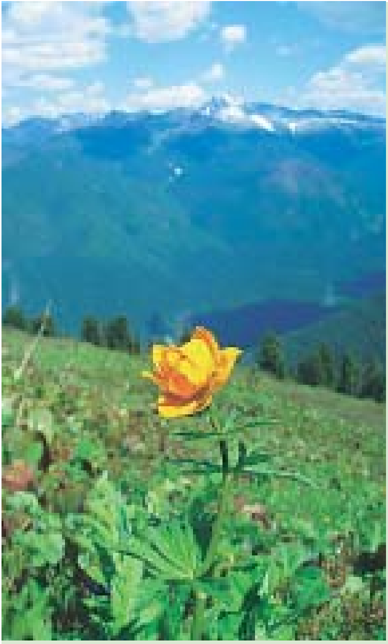
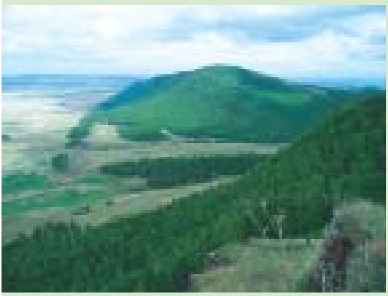


METHODOLOGICAL RECOMMENDATIONS FOR BOTANIC GARDENS ON THE REINTRODUCTION OF RARE AND THREATENED PLANTS



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MOSCOW
2008

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Methodological Recommendations for Botanic Gardens on the Reintroduction of Rare and Threatened Plants.
Tula: Grif & Co, 2008. — 53 pp. with photos.
ISBN 978-1-905164-28-8

Reviewed by
N.V. Trulevich, Dr. of Sci. & Yu.K. Vinogradova, Dr. of Sci.

Under the Editorship of
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Methodological approaches for the reintroduction of rare species of plants are given. Issues of terminology and the selection of objects for reintroduction are discussed. Features of preliminary studies, selection of initial material, determination of natural habitats, and processes for the creation and monitoring of reintroduced populations are described. Special attention is paid to the necessity of providing genetic diversity within created populations and thorough documentation of conducted works. A separate section is dedicated to the method of reintroduction of plant communities (agrosteppe creation method). Examples of practical experience of reintroduction of rare species in various regions of Russia (Bashkortostan, the Far East, Irkutsk and Vladimir regions) are given.

Translation from Russian into English by
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Translation edited by
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This publication was prepared with the support of Botanic Gardens Conservation International. Some materials presented were prepared by the Programme of the Russian Academy of Sciences «Biodiversity and dynamics of gene pools».

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Content

INTRODUCTION

The threat of extinction of wild flora and its habitat is rapidly growing throughout the world. It is known that preservation of individual species and plant communities *in situ* is preferable to preservation *ex situ*. However, in many regions the scale of ecological community devastation does not allow the preservation of plants under natural conditions. Therefore, the reintroduction of some species in conservation areas for future recovery and the reconstruction of entire communities are to be regarded as potential measures for saving plants threatened by extinction.

Reintroduction as the means of recovery of plants and plant communities or the enhancement of their vitality remains a challenge. It includes long-term, costly, and time-consuming work and often cannot guarantee 100% success. Thus, reintroduction should be considered when all other tools for conservation and recovery of populations *in situ* have failed [Valee et al., 2004].

By conducting more thorough studies on natural populations it is possible to learn ways to conserve threatened species *in situ* through the protection of habitats (conservation of territory, restrictions on cattle pasture and man-generated activities, elimination of fires, weeding, etc.) or by initiating measures for recovery of species and entire populations. Certain methods are targeted at promoting or renewing natural processes to increase the vitality of a particular population. These measures include hand pollination, burning, thinning, cleaning and disturbance of the topsoil, etc. These tools are advantageous because they are economical, cause minimum disruption to evolutionary processes, and are most likely to stabilize populations [Hogbin, Peakall, 2000].

However, for populations characterized by very low numbers or species with a small number of existing populations these measures can be ineffective and even unsuitable. In these cases one has to resort to reintroduction.

Over the years, the former USSR and now Russia have accumulated an enormous amount of informative material concerning the reintroduction of plants. Foresters have great experience with artificial reproduction and the transfer of populations of precious species. Our country conducted numerous research projects on the creation and recovery of stocks of fruit, berries, livestock feed and medicinal plants. They were published in the journal “Lesnoye khozyaystvo” (“Forestry”) (1960–1980), and in proceedings of conferences on research, protection and revegetation [Ohrana i vosstanovlenie…, 1978; Dostizhenija i perspektivy…, 1986].

In 1970-80s in the Moscow region, experiments on the creation of artificial populations of *Hepatica nobilis* Mill., *Pulsatilla patens* (L.) Mill., and *Pulmonaria angustifolia* L. in their former habitats were carried out by G. P. Rysina [1981, 1984]. Many articles are devoted to artificially developed, self-renewing populations located in specially protected territories and in sites of natural biotopes in botanical gardens [Muzharaja et al., 1983; Nechitaylo, Pogrebennick, 1986; Nechitaylo, Kucheryavaya, 1990; Andreev, 1991; Gruzdeva, 1995; Radchenko, Tomilova, 1996; Shatko, Mironova, 2000; etc.].

In 1982 V. L. Tikhonova and her disciples worked according to a specially developed methodology for studying, propagating and creating artificial populations of 32 protected plants in forest parks of Moscow and the Moscow region [Tikhonova et al., 1995; 2000; Tikhonova, 1999; Tikhonova, Belovodova, 2002]. More than 160 artificial populations were created of *Trollius europaeus* L., *Iris pseudacorus* L., *Dianthus fischeri* Spreng., *D. superbus* L., *Campanula persicifolia* L., *C. latifolia* L., *C. trachelium* L. and others. Until now 150 populations have been preserved and developed.

In recent years interest in reintroduction of rare and threatened plants has considerably increased. The number of related publications has grown. Often however, they are scattered in journals and editions not available any longer and basic methodological principles for the reintroduction of plants, especially rare species are underdeveloped. Terminology used in this field is complex and a general protocol for documenting how to conduct operations has not been worked out.

Important guidelines include the following: 1) Handbook for botanic gardens on the reintroduction of plants to the wild published by BGCI [Akeroyd, Wyse Jackson, 1995]; 2) Methodological approaches to translocation of plants [Korovin, Kuzmin et al., 2001]; and 3) Guidelines for the translocation of threatened plants prepared by Australian botanists [Valee et al., 2004].

For methodological recommendations in the Russian context, we tried to consider the accumulated experience of both domestic and foreign workers regarding the reintroduction of plants, as well as basic publications in this area. In addition, we tried to consult and draw on experts with a vast and longstanding experience in reintroduction programmes who have achieved successful and practical results in their studies. These experts have contributed to Section 4 of this handbook: L.M. Abramova and A.A. Muldashev, “Reintroduction of rare species in the Republic of Bashkortostan”; S.V. Nesterova, ”Reintroduction of *Aristolochia manshuriensis* in the Russian Far East”; V.Ya. Kuzevanov, “Reintroduction of *Allium altaicum* in the Lake Baikal Region” and R.Z. Saodatova, “Reintroduction of *Primula veris* in the Vladimir Region”.

Authors express their gratitude to N.S. Mergelov of BGCI for assistance in working on methodological recommendations.

1. RUSSIAN BOTANIC GARDENS AND REINTRODUCTION OF PLANTS

Botanic gardens throughout the world grow more than 80000 species of plants, 1/3 of all species described to this day [Wyse Jackson, 2001]. The role of botanic gardens in the conservation of plants has even grown since the Conference of the Parties to the Convention on Biological Diversity adopted the Global Strategy for Plant Conservation (GSPC) [2002]. This unique strategy aims to halt the current and continuing loss of plant diversity. The most important element of the GSPC includes concrete goals for implementation by 2010. Of particular importance for botanic gardens is GSPC Target 8 stipulating that 60% of threatened plant species must be represented in publicly accessible *ex situ* collections, preferably in the country of origin, and 10% of them should be included in recovery and restoration programs.

In order to clarify the actual situation concerning the protection of rare species *ex situ*, in 2002 the Commission on Rare and Endangered Plants of the Russian Council of Botanic Gardens started to compile the database on rare plant species represented in the Russian flora and grown in botanic gardens of the Russian Federation. The database reveals that of the 461 species of angiosperms, gymnosperms and ferns included in the Red Book of the Russian Federation [Krasnaja Kniga... , 1988], 249 species (54%) are grown in Russian *ex situ* conservation centers. Thus, Russian botanic gardens are close to reaching their main goal as stated by the Global Strategy for Plant Conservation of conserving 60% of threatened species *ex situ* nationwide [Rastenija Krasnoj knigi…, 2005; Gorbunov, Orlenko, 2005].

As for plants included in reintroduction programmes, until recently we did not have any information. In 2007 the Russian Division of Botanic Gardens Conservation International together with the Commission on Rare Species of the Russian Botanic Gardens Council conducted a questionnaire regarding the reintroduction of plants conserved in botanic gardens. Fifteen institutions provided information. We provide some examples below.

The botanic garden of Ufa Scientific Center of Russian Academy of Sciences is working on the reintroduction of populations of 9 species, including 2 endangered species of the Red Book of the Russian Federation (*Paeonia hybrida* Pall. and *Hedysarum razoumovianum* Fisch. et Helm) and 7 species of the Urals regional Red Book. The reintroduction of *Rhodiola iremelica* Boriss. was successful; sustainable populations were created. Work with *Allium hymenorhizum* Ledeb. also shows promising results.

The Stavropol Research and Scientific Agricultural Center applies the method of agrosteppe creation developed by D.S. Dzybov [2001]. It is a speedy method for the accumulation of a natural steppe flora gene pool *ex situ*. It also provides a method offering stable ecological conditions for the survival of populations of reintroduced rare and endangered steppe species. The method comprises collecting a multi-species mixture of seeds from a natural ecological community and sowing them in fallow land. A recovered plant community of 10,4 hectares including rare plants was created in the test site of Stavropol Research and Scientific Agricultural Center. Rare plants include 4 species of the Russian Federation Red Book (*Iris notha* Bieb., *Orchis tridentata* Scop., *Stipa pulcherrima* C. Koch, and *Paeonia tenuifolia* L.) and 5 species of the Red Book of Stavropol region [Krasnaja Kniga Stavropolskogo Kraja, 2002]. Most of the species in this community are sustainable and recover by seeds. The same method is implemented in the Botanic Garden of Southern Federal University working with 7 species of the Red Book of Rostov region. All 7 species are characterized by sustainable seed reproduction during a 5–20 year period.

Since 1992, the Botanic garden and Institute of the Far East Department of Russian Academy of Sciences has been reintroducing the endangered species *Aristolochia manshuriensis* Kom. Populations were planted in 3 selected sites; by 2007, only one of them survived.

In 2001–2003 the Botanic Garden of Irkutsk State University created several reintroduced populations of *Allium altaicum* Pall. (included in Red Book of Russian Federation) in areas of 50–150 m^2. The populations were founded within two specially protected natural territories; they are sustainable, characterized by self-sowing and population expansion.

The list of species reintroduced by botanical gardens in recent years comprises 85 species, 20 of them included within the Red Book of the Russian Federation. Other species are referred to regional levels of protection or are not considered to be rare. In the nearest future various botanic gardens in Russia are going to develop reintroduction programmes for further rare species. However, as mentioned earlier, the lack of a general methodology on how to conduct reintroduction programmes is a major impediment in the success of these activities.

Reintroduction of rare species should include the following steps: 1) preliminary research — collecting detailed information concerning the biology of reintroduced species; 2) field investigations — studying the structure and ecology of existing *in situ* populations; 3) reproduction of the material *ex situ*; 4) preparation of habitat for artificial populations; 5) creation of artificial populations and 6) monitoring of reintroduced populations. The basic requirements at all stages include strict documentation of the conducted works and representation of the genetic diversity of the reintroduced material. One simply can not reduce the work to sowing material *in situ* without observing the future of the created population.

The second important issue of reintroduction concerns the selection of species for reintroduction. Botanic gardens should set species within the 0, 1 and 2 categories of the Red Book of the Russian Federation as priorities. Reintroduction of rare plants should follow the rules imposing minimization of losses to natural populations. Consequently, the Commission on Plant Conservation of the USSR Council of Botanic Gardens worked out "Rules for collecting rare and endangered plant species" [1981]. "Methodology for observations on valuable populations of species listed within the USSR Red Book" [Denisova et al., 1986] can also be useful while planning and conducting preliminary field work.

Another challenge lies in the lack of legislative support. It is clear that reintroduction is worth being undertaken in specially protected natural territories. However, officially in such territories reintroductions are prohibited. Nevertheless, some conservatories have testing sites where reintroduction projects can be conducted.

Reintroduction of rare and endangered species should be part of coordinated conservation action; the coordination of such work should be carried out by the Russian Botanic Gardens Council. The Strategy of Russian Botanic Gardens for the Conservation of Diversity of Plants [Strategia botanicheskih Sadov, 2003] states that:

"Work on reintroduction (repatriation) must be coordinated by the Commission on Rare Species of Plants of Russian Botanic Gardens Council which:

- Considers proposals on reintroduction;
- Gives permission for works;
- Collects information on monitoring of renewed populations and puts it in the integrated database;
- Fosters preparation of methodological recommendations on reintroduction".

However, most of the above stipulations are not followed currently. It is high time that a coordinated reintroduction work programme is developed with an emphasis on species listed in the Red Book of the Russian Federation which is followed by all Russian botanic gardens.

Botanic gardens have unique capacities for implementing the reintroduction of plants. They are often the only regional institutions possessing detailed documentation on collections of living plant material and banks of seeds and meristems. Botanic gardens have the required infrastructure and tools for propagating vegetation as well as experienced scientific staff, who can carry out practical tasks on reintroduction. Equally important, botanic gardens in Russia, in a global effort to strengthen plant conversation, should make their expertise and know-how in the development of recovery programmes as well as related data and information available to a wider pool of experts, e.g. through the periodical update of their data on BGCI's global databases. This could include the incorporation of data on living collections in the BGCI PlantSearch databases, used to monitor the achievement of GSPC Target 8 worldwide. It is hoped, that this work will enhance methodological guidance for the development of reintroduction programmes for a wide range of botanical institutions within and outside Russia.

2. METHODOLOGICAL ISSUES OF REINTRODUCTION OF RARE AND THREATENED SPECIES

2.1. Terminology

Numerous terms refer to the creation of artificial populations in natural biotopes: resettlement, reintroduction, restoration, repatriation, reacclimatization, naturalization, introduction, translocation, restitution, recovery, etc. [Luks, 1981; Sobolevskaya, 1981, 1983; Korovin et al., 2001; Tikhonova, Belovodova, 2002]. Often the same authors use different terms in their work. "Reintroduction" is the most frequently used term, but it implies various meanings. In a broad sense it is used in methodological documents of IUCN concerning reintroduction and translocation [IUCN, 1987, 1998]. The Commission on Rare and Endangered Species of the Russian Botanic Gardens Council recommends the following definitions of the terms:

Introduction — introduction of plants (genera, species, subspecies, varieties and forms) into a given natural or historical area where they have not grown before, or their transfer into the local flora [Ponjatija, terminy, metody…, 1972; Lapin, 1972].

Reintroduction — an attempt to establish a species in an area which was once part of its historical range, but from which it has been extirpated or become extinct. [IUCN, 1998].

Repatriation — returning plants from *ex situ* environments to their former natural habitats [Sobolevskaja, 1981].

Translocation — deliberate and mediated movement of wild individuals or populations from one part of their range to another [IUCN, 1998].

Restoration — recovery or strengthening of a species population under threat of extinction [Tikhonova, Belovodova, 2002].

2.2. Selection of species for reintroduction

Reintroduction is a labor intensive, time consuming and long-term process. Thus, a main role is played by a well-thought selection of species for reintroduction to ensure high probability of survival. Priority objects for Russian Botanic Gardens should include species of 0, 1 and 2 categories of the Red Book of the Russian Federation. Species which do not grow in specially protected natural territories should also be dealt with as a matter of priority. Selecting species also requires accounting for the economic, cultural, scientific and other values of plants. Criteria for the reintroduction of plants can be summarized as follows:

- Species of plants included in the Red Book of the Russian Federation;
- Species of plants included in the Red Books of IUCN and federal constituents;
- Species of plants characterized by useful properties and having important economic value (food, medicine, technical, decorative, etc.);
- Species of plants having cultural and historical value.

2.3. Preparation

The preparation step includes planning the project and collecting information concerning the targeted plant species.

The plan should incorporate all events from the beginning up to the end of the project. It is necessary to sort out the optimum working group and stipulate certain tasks for all project participants, and to assess the financial, material and time capacities for the project's implementation. Besides detailed research on taxonomy, genetic modification, reproduction, distribution and ecology of species the plan should include practical issues concerning vegetation growth, reintroduction and subsequent maintenance of the created populations. There is a need to define the type of material for reintroduction, approximate amount of seed and sowing material required, as well as sources of the material (natural populations, collections of the garden and other botanic institutes). It is also necessary to plan a schedule for the project.

Preparation research requires collecting a maximum amount of information on the reintroduced species. It demands reviewing the literature and existing herbaria data on:

- Taxonomy and systematic status;
- Morphological characteristics and variability;

• Historical and existing geographical distributions (habitat and certain locations);
• Ecological characteristics;
• Species biology (life span, morphogenesis, reproduction features, etc.).

2.4. Research on *in situ* populations of the target species

Detailed research on wild growing populations (if there are any) plays a key role in obtaining successful reintroduction. Research can reveal biotic and abiotic requirements of plants within environments, reproduction mechanisms, reproductive biology, symbiotic links (i.e. with mycorrhizae, pollinators), pests and diseases. Field investigations can help identify species which fill the niche of the threatened or extinct species under consideration, predict the species role within certain communities and, what is more important, find the reasons for its risk or extinction.

There are different reasons for the collapse of plant communities and the extinction of plants. Many of them are well considered by O. Frankel and M. Soule [1981] who regard human influence as the main factor for plant extinction. They also enlisted such detrimental biological factors as competition, foraging of plants by wild and domestic animals, diseases, space limitations and reproductive isolation leading to genetic erosion and loss of genes. Moreover, habitats are damaged as a result of geological processes, climate change and natural disasters.

L.B. Denisova, L.V. Nikitina and L.B. Zaugolnova [1986] recommended good methods for statistical, ecological and biological research on populations of wild growing plants.

As a minimum, programmes researching the status of natural populations of rare species should include:

Determining the ecology of populations within a habitat or part of a habitat and the characteristics of ecotopes and plant communities;

Calculating the number or average density of populations, and for large populations, evaluating the density in various parts of the population;

Studying life-forms, ontogenesis, and the range in age and group size of the plants;

Describing the morphometric characteristics of plants;

Recording the age spectrum of populations or the plant group size distribution within testing sites;

Describing the mechanism of population renewal and assessing the seed efficiency of specimens.

When studying plants *in situ*, it is necessary to pay special attention to their general state and appearance, including the external properties of seeds, presence of pathogenic organisms and parasites, damage caused by insects, and presence of herbivorous animals. Flowers and other parts of plants having abnormal development can point to signs of inbreeding, especially in small populations.

Besides a descriptive documentation of the plants on site, every population of rare and endangered species as well as individual plants should be photographed and their location should be specified on the plan.

It is also possible that detailed research on natural populations may indicate that reintroduction is less urgent than originally thought and that a less costly means to renew populations is warranted.

2.5. Collection of material for conservation *ex situ* and for reintroduction

Material for conservation and reproduction *ex situ* is collected during *in situ* investigations. A main role is played by using correct procedures for collection of plant material since proper sampling defines success of reintroduction of rare species of plants. This procedure should ensure the maximum collection of existing genetic variability. Any data on the systems of reproduction, genetic variability and species biology must be taken into account. Samples for conservation *ex situ* should be collected throughout the habitat and the genetic storage for the reintroduction ought to be located as close as possible to the site selected for reintroduction.

In theory, high quality material for reintroduction should represent at least 95% of the genetic variability of populations [Akeroyd, Wyse Jackson, 1995]. If the species is present in many populations, it is necessary to cover as many of them as possible, though for the majority of rare plants, sampling from more than 5 populations is quite representative.

When a population consists of more than 50 specimens, seeds should be collected from 10–50 (or more) randomly selected plants, depending on the amount of seed per plant. Depending on the number of seeds collected, one should decide whether it is worth reproducing seed of the species *ex situ*. In case there are enough seeds, reproducing the species *ex situ* is not necessary. A sample of about 5000 seeds from a population of 50–100 plants forms a sufficient basis for reintroduction; however, if the population size is smaller or the amount of seeds is less, seed reproduction is required.

When a population has less than 50 specimens, seeds should be gathered from each fruit bearing plant. It is very important not to damage such populations; collect no more than 20% of the seeds even if the population faces the threat of immediate extinction, for example, due to construction of an industrial facility. Where species are present in several isolated populations, we recommend harvesting seeds from all of the populations. If populations are spread out, we suggest collecting about 10–20 samples that reflect the ecological and geographical diversity. Every specimen must be labeled and stored separately.

The problem of insufficient numbers collected from small populations can be solved by collecting seed from the same populations repeatedly in different years. Collection over extended time periods can also be useful for populations of plants with prolonged blossoming and fruiting periods.

One should avoid collecting samples of plants infected by pathogenic organisms, especially viruses. To prevent contamination, collected samples must be isolated during storage and cultivation.

If seeds cannot be picked, but the plant propagates vegetatively one should collect bulbs, bulbils, corms, tubers, rhizomes, sprouts etc. Material should be picked from at least 10–15 specimens growing in a 100x100 m area, or even a smaller area.

2.6. Reproduction of material *ex situ*

The development of recovery for natural populations of plants requires a high quantity of fully developed genetic materials of the species (seeds, sprouts, cuttings, etc.). Natural populations of wild growing species are usually heterogeneous at both intra and inter-population levels. Artificial populations created by reproduction of the material *ex situ* should also be heterogeneous. Therefore, for reproducing species *ex situ* for return *in situ*, representative material must be diverse initially and genetic diversity must not be allowed to be eliminated in the process of reproduction.

For mass reproduction of initial material for reintroduction, special collection sites should be created which can provide enough vegetative plants or seeds. These sites should also form the basis for thorough research on plants *ex situ*. Isolated sites with very similar ecological conditions to those of the districts of reintroduction are the most suitable for these purposes. A special isolation zone surrounding the site should be no less than 100 m in width. Samples of seeds picked from each plant *in situ* are sowed separately. The mature plants are registered by one number in order to provide the maximum number of maternal genotypes during reproduction. If possible, samples are divided and to prevent loss of some genotypes, not all seeds are sowed at the same time.

The size of sites required for seed reproduction are defined approximately and depend on the particular biological morphology and seed efficiency of the species and on the assumed volumes of material required for reintroduction. For example, reproduction of narrow-leaved tussock grass requires rather small sites while bush-like, broad-leaved, or procumbent plants need considerably bigger territories. Reproduction sites should provide optimum regimes for the growth and development of plants, exclude possible contacts among plants, and be designed so that necessary conditions for plant attendance, security and collection of seeds are ensured.

Principles and methodologies for the creation of seed plantations of woody plants and for the organization of seed reproduction have been published [Nekrasov, 1971; Kronit, 1973; Rjabova, 1992; etc.]. The above publications mention recommendations that can be used for reintroduction work. However, practical implementation of these recommendations is possible only in botanic gardens having large acreage. In case of insufficient land, an option is the creation of introduced plantations within the areas planned for reintroductions.

Introduction involves detailed studies of the biological properties of plants following generally accepted methodologies. Special emphasis should be put on studying ontogenesis, possible ways of vegetative reproduction (division, cutting, inoculations, tissue culture) and reproductive biology (system of reproduction, mechanism of pollination, biology of blossoming and seed set, germination, dormancy and ways of its removal, conditions and duration for the preservation of vitality, etc.) [Metodicheskie ukazanija …, 1980].

The question under discussion is the wisdom of using plants grown for a long time in an artificial environment with the ultimate purpose of reintroduction. Plants in long-term cultivation can face significant genetic changes (genetic erosion, inbreeding, hybridization, deliberate and unintentional artificial selection).

V.I. Nekrasov [1980] noticed that in new, non-natural growing conditions mutative genetic variability is preserved and accumulates due to factors that promote selection of genes that are usually eliminated by natural selection and that the direction of natural selection is also modified. This results in the formation of populations that are different from the natural ones, i.e. introduction populations. This material can be used for reintroduction only with great caution. No doubt, it cannot be used for strengthening existing natural populations because it may cause unpredicted genetic consequences. At the same time such plants can be rather useful for studying biological properties of the species.

However, in the case of extinct or endangered species (0 and 1 categories) the only way to conserve the species is to create artificial populations of plants, which may be grown in botanic gardens for a long time. It is recommended to use the maximum possible number of sources of material, including those from other botanic gardens and introduction institutes. It is clear that material must be thoroughly verified in terms of taxonomy since identifications of plants from collections often contain mistakes. It is also recommended to study genotype variability by molecular methods (also for plants reproduced by tissue culture). Synthetic artificial populations using this type of material should be isolated from natural populations of the species.

2.7. Determining sites for reintroduction

In determining sites for plant reintroduction, the main focus must be placed on defining the former distribution borders and historical habitat of the species. Projection of the geographical habitat onto distributions of attributes (climatic, soil, geomorphological, etc.) can characterize the modern general ecological environment within the habitat and sometimes within specific separate sites. Reintroductions are satisfactory only if populations have recently disappeared from the natural flora. In these cases, succession processes (including human-generated ones) have not yet covered the whole habitat and natural patches remain or they have not yet caused considerable changes of the habitat. In these cases certain sites for reintroduction can be defined based on regional field investigation data.

Great difficulties are faced when species became extinct long ago or it is not known if they ever existed in the natural flora. Even with an approximate re-establishment of the historical geographic habitat, it is impossible to define the ecological habitat of these species with adequate accuracy and, subsequently, determine sites for reintroduction. During a long period of time, natural (and human-generated) succession processes lead inevitably to considerable general changes of the environment. In such cases determining reintroduction sites can be based on research on the biology and ecology of species *ex situ* [Korovin et al., 2001].

It is necessary when determining sites, first and foremost, to rely on analyses of data from previous field investigations, as mentioned above. The site's ecological characteristics should correspond to the ecological requirements of the taxon and there should be sufficient area within the sites to provide self-renewal and possible expansion of populations.

Priority in the search of sites for reintroduction should be given to those sites where the particular species grew in the past. However, sometimes such sites can be inappropriate due to the impossibility of neutralizing factors causing the original extinction of populations. In addition, if the site is populated by endangered species, any intervention in the ecological community can influence it negatively. Sites located close to settlements are unsuitable. The best choices are sites in protected areas, for example, in protected areas of reserves. Botanic gardens that have sites of natural vegetation can reintroduce plants, whose historical habitat cover the territory of the garden or are relatively close to it.

The number of sites for reintroduction of the species is determined by the general goals of the project and existing opportunities. They can be limited if landscapes of the historical habitat of the species have significantly changed. Some sites may be selected for distribution of valuable material from different places in order to prevent its loss or the failure of reintroduction in general. In other cases, especially when time and human resources are restricted it is rational to focus on one site.

2.8. Creation of reintroduced populations

The important issue in the formation of reintroduced populations is to determine the minimum number of plants required for a vital population. Until now this has been subject of arduous discussion among population biologists. They consider the effective number of specimens necessary for short-term survival of a population to be no less than 50 plants. Long-term survival of a population and continuous development of adaptations requires about 500 specimens [Soule, 1980; Frankel, Soule, 1981].

B.M. Pavlik [1996] states that the minimum number of specimens depends on many factors including the duration of the life cycle, the living form, system of reproduction, seed efficiency, ability for vegetative propagation, etc. For instance, he considers that perennials require a minimum of 50 specimens and annuals, 2500 specimens.

In practice if resources are sufficient it is better to grow as many plants as possible. If one fails to get enough plants during one year of reproduction it is necessary to make additional plantings over several years.

Before planting in natural conditions, plants should be prepared. Material grown in containers is preferable because it takes root better. Before transplanting, plants must be examined by a plant protection expert in order to avoid bringing pests and diseases into the reintroduction site. It is necessary to cut flowers and fruits off the plants and probably trim them.

In the beginning a preliminary plan of planting should be made. One can place plants in an arbitrary order, but it would complicate searching for them during ongoing monitoring. Therefore, it is better to place them in wavy parallel rows. When establishing plants reproduced by vegetative propagation, it is important to place plants of one clone in different areas in order to increase the efficiency of cross-pollination. Agronomic techniques (watering, mulching, fertilizing of soil, tying up of tall plants) are conducted after planting.

Planted species are labeled with the number given at the stage of reproduction in order to follow its future progress. It is better to use metal labels which are tied to the plant, dug near it or placed in a peg close to it.

Information on the date, provenance and location of sowing of each plant is registered and put in a schematic map of the site. Some plants and the site are photographed. If it is possible plants are regularly attended during the first vegetation season.

2.9. Monitoring of reintroduced populations and assessing success of the reintroduction

Monitoring of reintroduced populations is a crucial part of reintroduction work. All or selected plants are monitored. Monitoring can be based on the same methods of observation for population of plants within the ecological community, which were recommended above for preliminary field investigations [Denisova et al., 1986]. It allows data to be collected for comparisons between natural and artificial populations. In planning monitoring procedures, one should minimize the human-generated load on the ecological community under consideration (pinpoint pathways for movements in the reintroduction site, have a rational frequency of monitoring, increase the percentage of visual observations, etc.).

Assessment of success of reintroduction work includes the following parameters:

- Percentage of survived specimens;
- Vigor and health of the plants;
- Time required for transfer of plants, for blossoming and for seeding;
- Viability of seeds;
- Presence of plantlets and juvenile plants.

The duration of monitoring plants with relatively short life spans should amount to about 3 years; for perennials, it is longer. For perennials, observations should occur once every several years. Australian botanists [Valee et al., 2004] split the criteria for assessment of successful introduction into short-tem and long-term:

1. Short- term criteria

- More than 70% of plants survive, providing genetic diversity of the population.
- The newly created population has characteristics similar to wild growing plants.
- Plants survive to reproduction stage, giving blossoms and fruit.
- The levels of reproductive crop productivity and vitality of seeds are close to those of wild growing populations.

2. Long-term criteria

- Emergence of seedling offspring.
- The number of specimens within the population is stabilized or increases.
- Adequate level of biodiversity, especially genotypes, is preserved during changing generations.

The main results of the experiments on species reintroductions and features of the methodology being used must be published in scientific journals. Unfortunately, nowadays there are very few publications for this type of work.

2.10. Requirements for documentation

All stages of reintroduction work should be well documented. If the species are enlisted in the Red Book of the Russian Federation, we recommend getting agreement concerning the proposed work from the Commission on Rare Species of Russian Botanic Gardens Council, and for species of regional Red Books from the regional councils of Russian Botanic Gardens Council. After termination of species reintroduction projects, we recommend submitting information on the work to these organizations in a special form which is given below. This information is for including in the database on the reintroduction of rare species. Gradually filling the form can help during the course of the experiment itself.

2.11. Form for submitting data on reintroduction for including in the data base [with changes according to Yu.A. Luks, 1981]

1. Generic and species names of the plant, Latin and Russian.

2. Family name.

3. Category of rarity (according to federal or regional Red Book).

4. Nature of activities carried out: reintroduction, repatriation, translocation, restoration (not applicable to be ruled out)

5. Origin of the initial specimen:

a) administrative and geographical location, name of location (if there is any) where the sample was originally taken for reproduction in order to perform further reintroduction or introduction;

b) brief characteristics of the ecological conditions of vegetation in the location;

c) the date (day, month, year) of the original sample *in situ*;

d) family and given name, position of the collector or introducer; name of institute on behalf of which sampling was conducted;

e) state of sample when taken (seeds, whole plants or their parts, roots, bulbs, bulbotubers, tubers, stem or root cuttings, zones of apical points for further division, top meristem etc.) and its phenological phase;

f) the number of plants (their parts or seeds) in initial sample;

6. Brief characteristics of the conditions for growing and reproducing plants of the original sample:

a) administrative and geographical location where the original sample was transferred for reproduction; name of institution that conducted mass seed or vegetative reproduction of plants of the original sample;

b) brief information on the main characteristics of the place where natural samples taken for reproduction were transferred: remoteness from the initial natural location, brief characteristics of ecological conditions, and whether the transfer was made within one region of beyond its borders.

7. Method of reproduction used at preparation of sprouts for reintroduction or introduction:

a) seed reproduction: the first seed generation (the first reproduction — R_1), obtained from seeds collected *in situ*, i.e. from seeds R_0; the second seed generation (the second reproduction — R_2), obtained from seeds collected from the plant of the first seed generation*;

* Plants of the third and successive seed generations should not be transferred: they can be used for future cultivation, mass reproduction for receiving planting stock for landscaping, biochemical and other scientific research, organization of industrial plantations etc.

b) vegetative reproduction (common ways or mass-clone reproduction *in vitro*).

8. Documentation of replacement of plants *in situ* during reintroduction of the species:

a) introduction number;

b) the date (day, month, year), season;

c) family and given name, position of the person in charge of transfer; name of institute on behalf of which transfer was conducted;

d) total number of plantings (specimens); state of plants at transfer (age, phenological phase of development, etc.);

e) schematic map of location for transfer of plants with name of habitat, number of block within the plan of forest or land, as well as the orientation inrelation to any relatively permanent structures at the location; GIS-coordinates, size of area where the transferred plants were planted;

f) characteristics of the habitat where the plants were transferred (elevation above sea level, slope, aspect, brief description of vegetation and soil, degree of lighting and humidity);

g) plan for planting the transferred plants (it is not published, but placed in a reintroduction register; one copy of the plan is attached to a submitted registration card, the other is submitted to the scientific achieves of the institute on whose behalf the transfer was conducted for permanent storage);

h) it is desirable to provide a photo of the transferred plants immediately after the transfer and during the first season of vegetation following the planting (general view and one or two of the sites in close-up);

i) labeling of the transferred plants (individual or group) by fixing special labels on plants, pegs or by digging in certain places; it is obligatory to show label locations in the plan of transferred plants;

j) numbers of herbarium samples and place of their storage.

3. REINTRODUCTION OF NATURAL PLANT COMMUNITIES (METHODS FOR THE CREATION OF AGROSTEPPES)

Since the 1970s many botanic gardens in Russia have been collecting rare and endangered species. Botanic gardens contain considerable gene pools of rare species *ex situ* including small division expositions or artificial populations within natural biotopes. Large botanic gardens have a vast experience of conserving populations of rare plants within model, artificially created, reconstructed communities. These reconstructed communities provide numerous opportunities for the use of gene pools of rare plants collected in botanic gardens. Artificial communities could include experimental models of artificial populations for developing the methodology of reintroduction, as well as providing synthetic populations of rare plants for their long-term preservation.

In Russia the first model plant communities were probably created in the Main Botanic Garden (Moscow) [Trulevich, 1991]. Since 1958 the Central Siberian Botanic Garden (Novosibirsk) has developed a methodological basis and started working on the creation of models of virgin taiga, oak woods and park larch forest by transfer of live plants from the nature, sowing of seeds and planting of seedlings. Artificial plant communities were created in 4 steps [Lubjagina, 1989]:

1. Studying natural plant communities and constituent species;
2. Selection and preparation of the site for creation of artificial plant communities;
3. Transfer of plants;
4. Observation of the process of formation of green cover.

Large scale works on modeling of steppe and forest communities were conducted since the 1960s in Stavropol Botanic Garden. During 25 years, four grass and six wood formations were created [Skripchinsky, 1981; Dzybov, 1988]. Parts of the forest (birch, oak and hornbeam, beech, pine, and fir) were accomplished by planting sprouts taken form nature and grown from local seeds. After the formation of a bed, grass cover was created (by turfing, planting and sowing of seeds). At first stages of work, steppe and meadow communities were mainly created by replanting of turf. This method turned out to be the wrong perspective. It is not ecologically friendly because it considerably eliminates elements of the natural vegetation. Moreover, the formed artificial communities do not have long life spans.

Long-term experimental research and applications in industry have proved the effectiveness of creating agrosteppes, as shown by the work of D.S. Dzybov.

Agrosteppes (agromeadows) are multi component plant communities renewed in places where they were completely eliminated by human-generated impacts (agriculture, pastures, construction, extraction of mineral resources) or as a result of natural events (landslides, human settlements, depressions, depletion of water reservoirs in arid districts etc.). Creation of agrosteppes systematically solves many ecological and environmental problems, as well as maintenance tasks [Dzybov, 1986, 1988, 2001]:

1) protection and active reproduction of populations of rare and endangered species in an ecologically friendly, adaptive environment that has maximum similarity to the natural parameters of the flora under conservation;

2) conservation of all biodiversity in fragments of zonal vegetation including rare and economically valuable relatives of cultivated plants (flower and ornament, medicine, food and other plant uses);

3) creation of permanent biomes with zoological components (mammals, reptiles, birds and insects) and formation of biophysical communities like natural steppes (prairies, steppes, forest steppes) and meadows;

4) protection of soil from erosion by forming natural plant cover;

5) extension of the area of steppe, meadow, forest steppe and similar types of vegetation by speedy recovery of pastures, degraded feed pastures and other bad lands;

6) creation of protected areas around reserves, national parks, botanic gardens or complex conservatories containing rare species of flora and fauna, making a barrier between agricultural and industrial landscapes and specially protected areas.

3.1. Maintenance of optimum ecological conditions for rare species

When reintroducing plant communities in order to preserve rare and endangered species one should anticipate:

1) developing a natural model of the plant community (steppes, prairies, meadows etc.) with close abiotic conditions to the place of reintroduction (principle of ecological conformity);

2) within a complex seed bank of the entire flora, reintroducing plants only in a manner that preserves the relative abundance of plant species, an indispensable condition for getting a representative model of the initial seed community in a new environment (the principle of floristic similarity);

3) creating equal conditions for all components reintroduced at the first stages of artificial community formation (height above the sea level, exposure, soil without weeds, fertilizers, herbicides, weeding etc.); moreover, it is possible that the number of protected plant seeds in a general sowing bank would be excessive, in particular, those with a low capacity for sprouting (for example, *Adonis vernalis* L., *Paeonia tenuifolia* L. etc.);

4) following a regime of sustainable resource use in the newly created plant community, thanks to which the donor ecological community will have been conserving populations of rare species for a long historical period (absolute conservation is unsuitable);

5) encouraging the appearance and reproduction of faunal components in the ecological community, while developing it for rare plants (refusing pesticides, chemical remedies, insecticides, etc.);

6) abolishing any type of hydro subsidies — individual watering, irrigation, and other agricultural meliorating tools conducive to a hydrological imbalance and a reduction in the life span of the community. Exceptions concern only water requiring species and groups.

The general principle of protection of rare species in agrosteppe associations implies a maximum compliance with conditions of natural functioning ecosystems, including the forms of resource use (disturbance of phytomass on the ground can lead to the regeneration of the initial natural community).

3.2. Determining a model of the initial natural ecosystem

One of the main stages defining successful reintroduction of rare plants within agrosteppe expositions in botanic gardens and other conservation establishments is the model of the initial natural ecosystem. The main criteria for determining the content of wild growing plant community are the following:

1) the maximum possible species richness for a zonal (belt) type of community defined in sample plots of 1 and 100 m^2 (for example, for meadow steppes of Stavropol the number of species (and maximum possible) in these areas is 35 (50) and 70 (95), respectively);

2) presence of dominate grass species (in steppe represented by rare species of *Stipa pennata* L., *S. pulcherrima* C. Koch, etc., as well as such community dominants as *Festuca rupicola* Heuff., *F. valesiaca* Gaudin, and *Koeleria cristata* (L.) Pers). A maximum amount of their seeds in the sowing mix guarantees that a sustainable representative model of the initial community will be formed;

3) genetic diversity of populations of rare species of plants in the plant community;

4) minimum representation of weed plants (abundance no more than *«sol.-un.»*[*];

5) diversity and abundance of Fabaceae species, since they constitute important sources of nitrogen fixation in soils when forming the plant community (for example, *Lotus caucasicus* Cuprian. ex Jus., *Medicago romanica* Prod., and species of genera *Amoria, Trifolium, Onobrychis*);

6) provision for the diversity of species of other than Poaceae and Fabaceae families; so called virgin land herbs (forbs) whose share in donor communities comprises 70–80% of total volume of the flora; for instance, *Aster bessarabicus* Bernh. ex Reichenb., *Stachys officinalis* (L.) Trevis., *Centaurea orientalis* L., *Dianthus ruprechtii* (Schischk.), *Inula aspera* Poir., and others.

3.3. Collection of seed grain

Seed grain contains a complex unregulated natural mixture of seeds with different admixtures (small parts of vegetative organs, particles of soil, weeds). This material forms a basis for the creation of a representative model of steppe (meadow) community during its reintroduction. Collection of the mixture can be carried out in different ways depending on the contractor's capacities: 1) by machine (harvester with air-supplier switched off); 2) by hand mowing followed by drying under the tent and thrashing.

The seed donor site, described in geobotanical terms (all types of measures including biomass) is divided in 2–3 equal areas. Seeds are collected from each area during certain time periods with intervals of about 25–30 days. The sowing grass mixture is gradually collected over different periods of time, reaping various species of seed (Table 1).

The first harvest, linked to the timing of steppe turf grass dominants, is the most important. The seed potential of these species in the sowing mixture

[*] Scale by German Botanist *Carl Georg Oscar Drude*, 1852–1933.

defines the representation, i.e. the degree of similarity between the created plant community and its donor — the pattern of the virgin land. Periods of harvest shown in Table 1 are to become more specific in the concrete areas of the projects, depending on differences in climate conditions and possible fluctuations of the environment in various years (droughts, spring frost etc).

Table 1. Approximate periods of seed mixture harvest in southern steppe regions of Russia

Plant communities	Periods of harvest		
	first	second	third
Desert-like arid steppes	15.V–05.VI	01.VII –10.VII	20.VIII–15.IX
Herb-bunchgrass steppe	05.VI–15.VI	01.VII –15.VII	25.VII–10.VIII
Meadow steppes	20.VI–30.VI	20.VII–30.VII	20.VIII–30.VIII
Mountain steppes	15.VII–20.VII	10.VII –15.VIII	25.IX–20.IX

Observations in natural ecosystems, steppes and meadows, as well as in experiments during the development of the agrosteppe method have established that the date of the seed reaping period (Table 1) occurs at the same time for different types of plants in the ecological community; for example, in the meadow steppe zone, 7–10% of species can be harvested in the first period, 50–80% in the second (2^{nd}–3^{rd} decade of July), and the rest (late summer plants) at the end of August. A considerable number of species are characterized by a long phase of dissemination: seeds ripen at the end of summer and stay on the plant through winter until the next year (*Agropyron pectinatum* (Bieb) Beauv., *A. desertorum* (Fisch.ex Link) Schult., *Verbascum lychnitis* L., *Stipa capillata* L., etc.). The main part of the flora, except secondarily blossoming species *(Lotus caucasicus* Kuprian. ex Juz., *Leontodon hispidus* L., *Poterium polyganum* Waldst. et Kit., *Silene densiflora* D'Urv. and others; more than 50 species) finishes the phase of fruiting and seed dispersal by the end of August, which is also true for shrubs *(Crataegus monogyna* Jaq., *Prunus spinosa* L., *Rosa canina* L., *Chamaecytisus ruthenicus* (Fisch. ex Woloszcz.) Klaskova, *Genista tinctoria* L., etc.)

Numerous long-term practical experiments conducted in many regions of the USSR and Russia show that the method of agrosteppe creation does not damage the donor ecology and is easily implemented through a developed mechanized strategy. In small territories, it can be performed by hand sowing of seeds onto the prepared land.

3.4. Pre-sowing preparation of soil

Before sowing, the soil should be loosened and must not contain vegetative weeds, potential rivals of sprouting steppe (meadow) plants. Ploughing at a depth of 16 (18)–20 (22) cm or with 2–3 times of harrowing (with disc harrow BDT-7) before winter is the best time of soil preparation for future agrosteppes with rare species of plants [Dzybov, 2001]. Sowing is preceded by cultivation of the soil. The best time for sowing in arid or sub-arid districts is during "February windows" or in the first ten days of April. Surface sowing of natural mixture of seeds is carried out by the spreader device for mineral fertilizers (1-RMG-4A, RUM-8, RUM-5). The unloading dozer window of RUM is set at the mark of "1" or "2", thus, fulfilling the norm for the sowing of seed mixtures (40–45 kg/hectare) and getting an even agrosteppe community. Then, the seeds are evenly spread in the field by tooth harrows "Zigzag" (in smaller sites by rake). After spreading, the sowed area is rolled by ring or flat rollers without any brake twice along the field diagonal. In this case seeds sowed in various depths of lumpy surface are finally embedded and receive the required contact with the soil.

3.5. Formation and monitoring of reintroduced plant community with rare species

The first year of the community

After sowing and forming an artificial community (agrosteppe, agro meadow, agro prairie etc.) the process of establishing various plant populations and the gradual penetration of faunal components into the new plant community begins. A quasi-natural biogeocenosis is formed in a new place [Dzybov, 1985]. The stages of formation into a sustainable ecosystem have characteristic features in floristic, ecological, demographic and other aspects (results of monitoring of this process are given below in a concise form).

Any soil in the form of arable land contains a certain stock of segetal and ruderal weed seeds characterized by an immense energy for sprouting, growth and development. In the first year during formation of an agrosteppe with rare species this storage of weed species in the soil creates a tense competition with steppe species for main ecological factors, namely, humidity, elements of nutrition, and lighting. Sprouts of wild growing (virgin land) species pass the stages of vegetation and development slower than weeds. There is a need to weaken some weed annuals and perennials. Rivals are weakened by mowing young grasses at a minimum height of 15–20 cm from the surface, two or three times the first summer after sowing. At this time the experimenter deals with a plant community in which species of the wild flora are in the form of seedlings and as juvenile and immature specimens chaotically spread over the field. Besides weakening weeds and optimizing the growing and developing conditions for steppe grasses and shrubs, mowing plants encourages the formation of micro groups and age differentiation of populations in the range of seedlings and young generative specimens.

In the first summer of plant vegetative growth, in the south of Russia in the agrosteppe the most frequently met weeds growing from the seed storage in the soil are the following: *Acinos arvensis* (Lam.) Dandy, *Alyssum turkestanicum* Regel et Schult, *Amaranthus blitoides* S. Wats, *Ambrosia artemisiifolia* L., *Anisantha tectorum* (L.) Nevski, *Arenaria serpyllifolia* L., *Bromus japonicus* Thunb., *Capsella bursa-pastoris* (L.) Medik, *Cardaria draba* (L.) Desv, *Centaurea cyanus* L., *Chenopodium album* L., *Daucus carota* L., *Echinochloa crusgalii* (L.) Beauv, *Echium vulgare* L., *Erodium cicutarium* (L.) L'Her, *Fumaria schleicheri* Soy.–Willem, *Galium aparine* L., *Medicago minima* (L.) Bartalini, *Microthlaspi perfoliatum* (L.) F.K. Mey., *Senecio vernalis* L., *Sinapis arvensis* L., *Trifolium arvense* L., *Veronica hederifolia* L., and *Viola arvensis* Murr.

It is very important for the soil not to contain seeds and roots of weeds such as *Elytrigia repens* (L.) Nevski, *Cirsium incanum* (G.S. Gmelin) Fisch. and some other malicious weeds of the agrosteppe. If one fails to avoid their penetration into the created community it is necessary to remove them in the first 2–3 years within a distance of 0,7–1,0 m around the populations of rare plants.

If the weeds are treated by 2–3 mowings, an established agrosteppe with rare species can successfully winter.

The second and successive years of the agrosteppe

A two-year old agrosteppe community is characterized by:

1) almost complete suppression of weeds by the competitive force of virgin land perennials, (3–4 types of weeds face the abundance of species);

2) the cover of soil at the end of June comprises 70 (90)%;

3) 95–97% of steppe perennials are in a generative form ("budding-fruiting"); rare species grown from seeds do not blossom yet, but plants transferred with soil pass through the whole development cycle and give fruits;

4) aspectivity of agrosteppe per season comprises 5–6 shifts (like the analogous natural steppe);

5) formation of above-ground plant biomass precedes penetration of the soil horizons by root systems; the bulk mass and volume of roots (90%) is located

in the 0–12 cm layer , while some stem roots (*Medicago romanica* Prod., etc.) penetrate down 20–30 cm;

6) the position of dominants, turf and other grasses: *Festuca valesiaca* Gaudin, *Koeleria cristata* (L.) Pers*., Phleum phleoides* (L.) Karst and others, is clearly set.

Being natural components of the agrosteppe after their sowing, protected species of plants start blossoming and fruiting at different times according to the species and their biological characteristics: species of feather grass, in 3–4 years; iris, in 5–7 years; orchids in 8–10 years; anemone, in 5–8 years; etc. Narrow-leaf peony enters a generative stage that lasts one to 15–20 years after planting.

We use a geobotanical description (made in May, 2007) as an example of the floristic content of a reintroducted perennial plant community containing rare species (Fig. 1). The age of the reintroduced plant community is 27 years and its areal extent is 100 m^2. The soils are sandy and poor with the humus content around 1,0% at the starting point of experiment. The slope has southern exposition and 8–10° steepness. Association of the agrosteppe: *Festuca valesiaca* + *Amoria montana* + *Stipa pulcherrima*; I level — 80 cm; II level — 60 cm (basic in biomass); III level — 30 cm; projected cover — 85–90%; actual cover — 3,8%. In the 100 m^2 plot, there were 71 species. At the time of description, plants of the population were in the following phases: vegetative — 14,1% of total number of species in the area; budding — 16,9%; efflorescence — 5,6%; blossoming — 35,2%; blossom fading — 12,7%; fruiting — 15,5%. The total generative stage of species of the community comprised 86%. Taking into account late summer blossoming and fruiting, this stage covered more than 90% of the agrosteppe species. The high percentage of species in the generative stage forms the basis of a self-supporting and self-reproducing agrosteppe community with rare protected plants, without human influence.

Besides rare species, the registered area also includes numerous representatives of various zonal grasses and turf steppe (Table 2). Therefore, rare and endangered species are in an optimal adaptive phytosphere without man-made impact, except strictly regulated regimes of influence. As was mentioned above, the experimental research showed the unsuitability of absolute conservation of the created community. One should mow hay and occasionally, moderate the pasture in reintroduced plant community. If this man-made influence is absent, steppe and meadow communities accumulate dead plant remains which have negative impact on the ecology. This process could lead to a transformation of the original model of the agrosteppe, by modifications to root-fall weeds, which are detrimental for rare species of plants.

Table 2. Content of 27-year old agrosteppe
(sowed in August, 1980; description in May, 2007)

Type	Abun-dance*	Species	Abun-dance
Grasses and sedges:		*E. stepposa Zoz*	*Sp2*
Briza media L.	*Sol*	*Falcaria vulgaris Bernh.*	*Sp2*
Bromopsis inermis (Leyss.) Holub	*Cop1*	*Filipendula vulgaris Moench*	*Cop2*
B. riparia (Rechm.) Holub	*Cop1*	*Fragaria viridis (Dush.) Weston*	*Sp2*
Carex michelii Host	*Sol*	*Galium ruthenicum Willd.*	*Sp3*
Elytrigia repens (L.) Nevski	*Sp2*	*Geranium sanguineum L.*	*Cop1*
Festuca rupicola Heuff	*Sp3*	*Hieracium echioides Lumn.*	*Sp3*
F. valesiaca Gaudin	*Cop3*	*Holosteum umbellatum L.*	*Sp3*
Koeleria cristata (L.) Pers.	*Cop1*	*Iris aphylla L.*	*Sp1*
Phleum phleoides (L.) Karst.	*Sp3*	*Jurinea arachnoidea Bunge*	*Sp3*
Poa angustifolia L.	*Sp3*	*Linum nervosum Waldst.et Kit.*	*Sp2*
P. bulbosa L.	*Sp2*	*Muscari muscarimi Medik.*	*Cop2*
Stipa pulchherrima C.Koch	*Cop2*	*Orchis tridentata Scop.*	*Sp1*
Legumes:		*Paeonia tenuifolia L.*	*Sp2*
Amoria ambigua (Bieb.) Sojak	*Sp3*	*Pastinaca armena Fisch.et A.Mey.*	*Sol*
A. montana (L.) Sojak	*Cop3*	*Pedicularis sibthorpii Boiss*	*Sp3*
Medicago minima (L.) Bartalini	*Sp1*	*Plantago lanceolata L.*	*Sp2*
M. romanica Prod.	*Sp3*	*P. media L.*	*Sp3*
Onobrychis arenaria (Kit.)DC.	*Sp1*	*Polygala caucasica Rupr.*	*Sp2*
Trifolium arvense L.	*Sol*	*Potentilla adenophylla Boiss.et Hohen.*	*Sp3*
Vicia angustifolia Reichard	*Sp3*	*P. argentea L.*	*Sp2*
V. tenuifolia Roth	*Sp1*	*P. recta L.*	*Sp1*
Other families (various forbs):		*Poterium polygamum Waldst.et Kit.*	*Sp3*
		Pyrethrum corymbosum (L.)Scop.	*Sol*
Achillea setacea Waldst. Et Kit	*Sp2*	*Ranunculus polyanthemos L.*	*Sp2*

* Scale by German Botanist Carl Georg Oscar Drude, 1852–1933.

Type	Abun-dance	Species	Abun-dance
Anemone sylvestris L.	*Sp2*	*Salvia tesquicola Klok. et Pobed*	*Sp1*
Arenaria serpyllifolia L.	*Sp3*	*S. verticillata L.*	*Sp1*
Campanula praealta Galuschko	*Sp3*	*Scabiosa ochroleuca L.*	*Sp2*
Centaurea orientalis L.	*Sp3*	*Stachys atherocalyx C.Koch*	*Sp3*
Cerastium holosteoides Fries	*Sp3*	*Teucrium chamaedrys L.*	*Sp3*
Cichorium intybus L.	*Sp1*	*Thymus marschallianus Willd.*	*Sp2*
Convolvolus arvensis L.	*Sol*	*Tragopodon dasyrhynchus Artemcz.*	*Sp2*
Dianthus ruprechtii Schischk.	*Sp3*	*Tromsdorfia maculata (L.) Bernh.*	*Sp2*
Dracocephalum austriacum L.	*Sp3*	*Verbascum lychnitis L.*	*Sp1*
Echium russicum J. F. Gmel.	*Sp3*	*V. phoeniceum L.*	*Sp1*
Erodium cicutarium (L.) L' Her.	*Sp1*	*Veronica chamaedrys L.*	*Sp3*
Eryngium campestre L.	*Sol*	*V. dentata F.W. Schmidt*	*Sp3*
Euphorbia iberica Boiss.	*Sp1*	*V. verna L.*	*Sp3*

One should note that the above given illustration does not represent the full diversity of the created agrosteppe. Dozens of other species can be encountered beyond the registered area. The total number of registered species in the experimental field of 2 hectares fluctuated in time from 127 to 131 including some steppe and forest-steppe shrubs: *Prunus spinosa* L., *Rosa canina* L., *R. pimpinellifolia* L., *Chamaecytisus ruthenicus* (Fisch. ex Wotoszcz.) Klaskova, *Genista tinctoria* L., and *Crataegus monogyna* Jacq.

Populations with a diversity of species are represented by the full age spectrum from seedlings to generative and old specimens and witness the appearance of many companion animals and, consequently, optimal pollination of plants.

The described methodology of creating artificial communities (agrosteppes, agromeadows etc.) by its technology and final results provides maximum similarity to natural plant communities and provides optimal conditions for the long-term preservation and reproduction of gene pools of rare and endangered species. The method is simple and available for botanic gardens and other introduction and conservation institutes, and is environmentally friendly and economically efficient.

4. PRACTICAL EXPERIENCE ON REINTRODUCTION OF RARE SPECIES

4.1. Reintroduction of rare species in the Republic of Bashkortostan

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4.1.1. Reintroduction of Rhodiola iremelica

During the last 20 years, rhizomes and roots of *Rhodiola iremelica* A. Boriss. *(Crassulaceae)* have been actively collected for their medicinal properties, analogous to *R. rosea* L. As a result, the species has been under threat of extinction and was included in the Red Book of the Republic of Bashkortostan. Now the species can be conserved only when special measures are taken, including reproduction *ex situ* (creation of nurseries, mass reproduction *ex situ*) and subsequent reintroduction into natural habitats.

Since 2000 the Botanic Institute and Garden of Ufa Scientific Center of the Russian Academy of Sciences, together with the Laboratory of Geobotany and Plant Preservation of the Biological Institute of Ufa Scientific Center of the Russian Academy of Sciences have been conducting research on *R. iremelica in situ*, in order to determine its distribution and the current ecological state of its populations (Fig. 2, 3). It has been found in recent years that the number of plants of this species has reduced dramatically and that most of the plants in existing populations (sometimes the whole population) are represented by old plants so greatly injured during the harvest that they do not produce blossoms or fruit.

Ex situ reproduction of *Rhodiola iremelica* involves planting material in the form of seeds and rhizomes (sprouts of caudex) annually from natural populations of the species. In 2001, two introduction nurseries were created in the botanic garden for conducting introduction research and accumulating mass seed: a “shade” nursery for the low-rising mountain forms growing naturally in forest and tundra plant communities and a “sunlight” nursery for mountain tundra populations growing in open sites. Today these nurseries include material from 17 *in situ* populations (about 1000 plants) (Fig. 4). Some plants are reproduced using methods of cloning microculture. Research is being conducted on the biology of the species and methods of reproduction *ex situ*.

Since 2001, work on the reintroduction of *R. iremelica* has been undertaken. Reintroductions were conducted in the mountain and forest zone of the Russian Federation characterized by a moderate cold humid climate (average annual temperature is 0,3–3,5°C; 40–50 frost-free days in the north and 80–120 frost-free days in the south; the hydrothermal coefficient in the north is 1,4–1,8, in the south, 1,0–1,2).

In determining sites for reintroduction we followed the principle that the planned sites should be quite remote from places of tourist interest and rarely visited. Another required condition was the presence of several habitats suitable for the growth of *R. iremelica*. The selected sites, on mountain ridge Irendyk (Uchalinsky District, two sites), on mountain ridge Irendyk (Abselilovsky District, two sites), and on mountain ridge Mashak, are located within the natural habitat of the species, where populations of *R. iremelica* are small (less than 100 plants) and not numerous due to the harvest. Reintroductions were mainly conducted in existing and planned specially protected areas. Sites were not treated unusually; during planting and sowing seeds, the upper layer of moss was removed and furrows were made.

Between 2001–2004, preliminary tests on the restoration of low-growing forms from seed were conducted in 3 sites on the Eastern slope of the Southern Ural Mountains, where the species is in threat of extinction. Seeds were sowed in moss furrows on rock shelves. In 2005, *R. iremelica* seedling establishment was checked in the sites sowed. Establishment turned out to be low (less than 1%) because of the severe natural conditions of *R. iremelica* habitat and the poor germination quality of the seeds that is characteristic of this species. Moreover, the furrows used for seed sowing were often washed away by water flows or filled with a thick layer of dry plant debris.

Since 2005, tests have been performed on the reintroduction of *R. iremelica* using material grown in nurseries of the botanic garden from seeds collected in nature. Seedlings (3–4-year plants) (Fig. 5) were sowed in lines filling cracks in rocks as well as in mossy rocks. More than 300 individuals were planted in 3 mountain peaks: ridge Irendyk (Uchalinsky district), mountain ridge Kurkak, and mountain ridge Irendyk (Abselilovsky district). Seedlings were planted in sites where the species had grown before and had almost being eliminated by harvest. An assessment of their condition in 2006 showed good establishment of plants (on average about 90%). The same year, populations were replenished by another 50 seedlings of *R. iremelica*. The seedlings were neither watered nor specially treated.

Moreover, in 2006, new experiments for creating reserve populations through planting seeds of *R. iremelica* collected in endangered populations of the Southern Ural were performed on the mountain ridges of Bahtau, Mayardak, and Northern Kraka. More than 1000 seeds obtained from the introduction nursery were planted. Vegetative propagations of *R. iremelica* using rhizomes were conducted and tested on the mountain ridge Mashak. 60 rhizomes were planted that were taken from plants living in natural populations (without digging the entire plant from the soil). In 2007 analogous experiments were conducted in the northern part of mountain ridge Krykty, where 50 rhizomes were planted. Research on the reintroduction of *R. iremelica* from seed was performed; 600 seeds were sowed in one of the peaks of mountain ridge Irendyk (Uchalinsky district) in the same place that 20 seedlings and 27 newly collected rhizomes were planted.

Since 2005, annual monitoring of reintroduced populations has been carried out (Fig. 6, 7). Monitoring assesses the germination of seeds and the establishment of seedlings. For example, in 2007 tests were performed on the reintroduction of *R. iremelica* through seedlings in mountain ridge Irendyk (Uchalinsky district), and in the mountains of Kurkak and Kuzgun-Tash in Abselilovsky District. The percent survival of plants sowed in 2005, 2006 and August 2007 was 52%, 56% and 89%, respectively. Additional plantings of *Rhodiola iremelica* grown in the botanic garden were carried out. Data on the morphometric characteristics of reintroduced plants was collected. In the same year (2007), evaluation of the 2006 reintroduction of *R. iremelica* through rhizomes in the mountain ridge Mashak was carried out. Over half (53%) of rhizomes were established; some plants blossomed by August, 2007.

A year after planting, 90% of seedlings were established; 2 years after planting, some of the plants sowed in harsher ecological areas had died; in 2006–2007 some of the reintroduced plants blossomed and produced seeds.

Therefore, experimental research showed proof of the concept for creating reintroduced populations with the help of seedlings and rhizomes. Vegetative propagation of *Rhodiola iremelica* with rhizomes appeared to be more economical, allowing flowers to blossom in a short period of time. The seed method of reintroduction is not as promising.

4.1.2. Restoration of populations of Paeonia hybrida

Paeonia hybrida Pall. *(Paeoniaceae)* is a relict species listed in the Red Book of the Russian Federation. There are only two habitats for the species in the Republic of Bashkortostan and the total number of plants is less than 100. Populations are located in the Bashkortostan Ural (steppes of the extreme southeast: Khaybullinsky district, "Popkov's lakes", and "Mount Sedlastaya"). The climate is continental, warm and arid. The average temperature is 1,9°C; the annual average precipitation is 300–320 mm; the sum of temperatures higher than +10°C is 2300°C; the frost-free period is 110–130 days; and the hydrothermal coefficient is 0,6.

The laboratory of Geobotany and Plant Conservation of the Biological Institute of Ufa Scientific Center of the Russian Academy of Sciences together with the Botanic Institute of Ufa Scientific Center of the Russian Academy of Science conducted biological and ecological assessments of natural populations, studied the species *ex situ* and analyzed the population structure *in situ*. They annually collected seeds from *in situ* populations and from the nursery of the Botanic Garden.

Since 2003, restoration (strengthening) of two small *in situ* populations has been on going. Biological features of the species (the slow growth rate and poor establishment after replanting), as well as the remoteness of the *in situ* sites from the reintroduction facilities (more than 600 km) prevent the use of any other method but seed reproduction for restoration of populations. From 2003 until 2007, six test sites were created. In restoration sites, 20x20 cm holes were dug and spaced 50 cm apart, turf was removed, and soil was tilled. 25–35 holes were made at each site.

A biological feature of *Paeonia hybrida* is that only newly collected seeds germinate because of seed hardness when seeds are old. Thus, 4–5 freshly collected seeds were placed in each prepared hole. In view of the arid climate of the habitat, the holes with newly planted seeds of *Paeonia hybrida* were regularly watered.

The annual monitoring of these reintroduced plants focused on the sprouting of seeds. Most sprouts appear a year after sowing. Plants develop slowly and even today have not reached their vegetative phase. A significant portion of the seedlings is eaten by earth-digging animals, who fill the holes. In general the population size has grown 1,5-fold.

4.1.3. Restoration of populations of Allium hymenorhizum

Allium hymenorhizum Ledeb. *(Liliaceae)* is a relict species listed in the Red Book of the Republic of Bashkortostan and considered a category 1 species (a species under the threat of extinction). There are data only on 2 habitats of this species in Bashkortostan, the total number of plants in all populations is less than 100. Populations are located in the Bashkortostan Ural (in steppes of the extreme southeast, Baymaksky and Khaybullinsky Districts, and in valleys of the Tanalyk, Sukrachka and Sapsal Rivers). The laboratory of Geobotany and Plant Conservation of the Biological Institute of Ufa Scientific Center of the Russian Academy of Sciences, together with the Botanic Institute of Ufa Scientific Center of the Russian Academy of Science conducted biological and ecological assessments of natural populations, studied the species *ex situ* and analyzed population structure *in situ*. They annually collected seeds from *in situ* populations and the nursery of Botanic Garden, where the introduced population (about 30 mature plants and about 50 self-sowed juvenile plants) was grown. *A. hymenorhizum* reproduces from seed quite successfully *ex situ*.

Since 2001, work on the restoration of two small populations and the creation of the reserve population have been accomplished. Biological features and good establishment of plants in cases of replanting allow the use of both seed reproduction and seedlings grown in the Botanic garden. In restoration sites (Fig. 8), 20×20 cm holes were dug spaced 40 cm apart, turf was removed, and soil was tilled. 25–35 holes were made in each site.

Since 2001, 25–30 3-4-year-old seedlings were planted annually in the habitat sites, as well as 100–200 seeds in the prepared holes. Plants and seed were moderately watered.

Since 2003, the annual monitoring of reintroduced plants has focused on seed germination and the survival and establishment of young seed plantlets (Fig. 9). Almost 100% of the plantlets became established (without accounting for the plants eaten by animals). Plants growing from seeds develop very slowly. Today, the plants in more than 200 holes have been preserved. Restoration of populations of *A. hymenorhizum* by plantlets is preferable compared to the seed method because in the arid conditions of the Ural, a very high percentage of sprouts die due to lack of moisture.

4.2. Reintroduction of *Aristolochia manshuriensis* in the Russian Far East

Author: S.V. Nesterova

Aristolochia manshuriensis Kom. *(Aristolochiaceae)* is a woody deciduous liana which creeps along tree stems up to 20 m. The species is listed in the Red Book of the Russian Federation as a category 1 species (species under the threat of extinction). In Russia this plant is located in the southwest of Primorsky krai, along the northwestern border of the habitat. Beyond the borders of Russia, this species spreads into the North Korean peninsula and Northwest China. It is a relict and an endemic of East Asia.

In three administrative districts of Primorsky krai, some of the species habitat locations are linked with the basins of the Malaya Borisovka, Nezhinka, and Anan'evka Rivers. The species does not grow in the preserves of Primorsky krai. Natural populations are characterized by a low level of genetic diversity; a considerable contribution to the reduction in species adaptation is made by the reduced gene flow of small populations [Nakonechnaya et al., 2007]. The structure and size of populations is negatively influenced by human activities.

The reintroductions of *A. manshuriensis* were conducted by the staff of Laboratory of the Far East Flora of the Botanic Garden-Institute of the Far-East Division of Russian Academy of Science. The work was targeted at creating reintroduced populations of *A. manshuriensis* in Primorsky krai in a site within the historical habitat, where the species had once existed before completely disappearing.

Reintroduction work included several steps.

Step 1. Examinating *in situ* species locations, studying the ecology and condition of populations.

Literature review and *in situ* field data on the growth of *A. manshuriensis* reveal strict plant community and ecological associations; the species grows in broad-leaved woods, in borders of forest glades and valleys of rivers. The species frequently occupies the lower parts of slopes with northern exposures. Mesophytic, it prefers moist well-drained soils and forms groups of 30–60, rarely 100 specimens, occupying sites of 100–250 m^2. Seed reproduction is low, as is the growth. There is no evidence that borders of existing populations are expanding. The plant is an important component of the biotope; its leaves are eaten by the relict butterfly *Atrophaneura alcinous*.

Step 2. Features of sprouting seeds and the individual development of plants were studied *ex situ*; new periods of ontogenesis and their corresponding age stages were identified [Nesterova, Nakonechnaya, 2007 a,b].

The seasonal cycle of development of *A. manshuriensis* is completed with fruiting. Fruit set is low, only 2–3% of ovaries (one ovary per flower) develop into fruit, a capsule that is up to 11 cm long and 2.5 cm in diameter. The actual seed productivity per fruit is 95% [Nakonechnaya et al., 2005].

Latent period of ontogenesis. Seeds are triangle, 7–9 mm long, 7–10 mm wide, and 1000 seeds weigh 29–31 grams; they do not need preliminary preparation, when sowed in the spring or autumn, 85–90% germinate.

Pregenerative period. Seedlings. Mass germination occurs in the second part of July. The main root grows downward and the powerful hypocotyl hook carries the two cotyledons together with the two first opposite leaves to the surface. The second internode is formed when the cotyledons fall off and the plant comes into the *juvenile age stage*. Sprouts grow and by the end of the first year, plants reach a height of 5–8 cm, the developing stem consists of

2–3(4) internodes, with leaves in an alternate arrangement. The main root reaches a length of 7–9 cm. The second vegetative period is characterized by the transfer of the plant into an *immature state*. Plants develop 2–3 lateral vines. Two-year-old plants reach 25 cm, the root forms branches and penetrates down to 20 cm. In the third growing season plants enter a *non-flowering state* which lasts for 4–6 years. Non-flowering plants are characterized by intensive growth and gradually appear as typical lianas. Vines grow and are able to twine around stems by their distal growth. Plants grow 1.2–2.0 m annually. The root penetrates deep into the soil.

Generative period. By the age of 6–8 years, plants enter the *generative stage* and blossom for the first time. Blossoming is not extensive and fruits do not form. Lianas continue to grow and reach heights of 10 m, the number of generative vines and flowers increase, and in 2–3 years generative specimens are fruiting annually.

Step 3. Reproduction of material in the nursery for reintroduction of species

Seeds collected from plants of *A. manshuriensis* in the Botanic Garden were sowed in the spring in nursery beds. When necessary, they were watered and weeded and their soil was loosened. Sprouts remained in the nursery for 2 years.

Step 4. Determining the reintroduction site

The selected site was located in the territory of Nadezhdinsky forest (50 km from the city of Vladivostok), 10 km west of the village Tikhy, on the left bank of the spring Zolotoy, not far from where it enters the river Pervaya. The site is located on the lower slope with northwestern exposure. Soils are moist, with moderate drainage. The site has a 2-level forest stand with shrub and grass cover. The canopy is open and the site is lighted with sun during the second half of the day. Special preparation of the site was not conducted.

Inhabitants of the village Tikhy witnessed this species growing in the basin of the river Pervaya in the 1950s.

Step 5. Transfer of plants from the nursery.

In July, 1992, 50 specimens of 2-year old *A. manshuriensis* seedlings were planted and located in a staggered order. Specimens between 15–20 cm in height were planted in an area of about 70 m^2. Plants were transplanted near trees, whose stems could later support the lianas.

Step 6. Monitoring the reintroduced population

The first monitoring was conducted in the summer of 1995 (three summers after planting). Examinations of plants showed root development and vine growth up to 20–25 cm in the stems of trees. Some of the plants died. After this, monitoring was conducted every 5–6 years.

According to the 2007 data, the reintroduced population consists of 15 non-flowering specimens of *A. manshuriensis* vines twisting around tree stems to a height of 1.5–2.0 m. Low light penetration through the tree canopy is detrimental for these plants since it delays the shift of *A. manshuriensis* into the generative state.

The first attempt to reintroduce this plant can be evaluated as a success. The work will be continued. The experience acquired, together with knowledge of all of the species ecological requirements will allow the selection of sites where plants would pass through their whole cycle of development and begin to fruit regularly.

4.3. Reintroduction of *Allium altaicum* in the Lake Baikal Region

Author: V.Ya. Kuzevanov

A botanic garden's introduction and subsequent reintroduction of a rare species (*Allium altaicum* Pall., *Liliaceae)* in 2000-2002 from damaged populations on the western bank of Lake Baikal provides an example of successful cooperation among three types of specially protected areas: the Botanic Garden of Irkutsk State University (S.G. Shvetsov, N.A. Puzanova, S.E. Kalinovich), the Near-Baikal State Natural National Park (A.E. Turuta) and the Baikal–Lena's State Reserve (N.V. Stepantsova, T.L. Troshkova). Each protected area has different tasks and objectives, but they all share a common goal, studying nature and the preservation of the Baikal as a part of the UNESCO World Natural Heritage List. *A. altaicum* is listed in the Red Books of the Russian Federation (Fig. 10), Irkutsk Region, and the Republics of Buryatia, Altai, and Tyva, as well as Mongolia, and has a 3rd environmental category. By the beginning of the 21st Century, Baikal populations of *A. altaicum* were highly damaged by tourists and locals due to the active exploration of banks of Lake Baikal.

Ecological state of populations. Studies revealed that in the Baikal–Lena's State Reserve and the Near-Baikal State Natural National Park, the species' plant communities differed in their degree of preservation and in their existing conditions. For example, in the Near-Baikal State Natural National Park populations can be grouped as following: **(1)** Those preserved with a normal or stable age structure, with the potential for self-preservation. Populations of rocky capes of Khoboy, Khara-Undur, Shunte and ravines of the mountain of Ulkhun in the North-Western bank of Olkhon Island can be referred to this group. **(2)** Those having a regressive age structure, but characterized by a sufficient number for self-reproduction, if negative external effects are eliminated. Populations in the surroundings of Sarma ulus, waterfalls of the Anga and Buguldeyka Rivers, and ravines of the capes Big Bell and Golden Cliff can be referred to this group. **(3)** Those having a very regressive age structure and low number of species making autonomous self-reproduction without seed replenishment from inside the population quite impossible. Populations in the surroundings of the waterfall of the River Goloustoynaya, capes of Big Censer, Small Censer, and districts (uluses) of Sakhyurta and Usyk can be referred to this group. **(4)** Disappeared populations, and those potential former habitats of *A. altaicum* with a preserved state and plant community structure favourable for active reintroduction of populations. The surroundings of villages Sharyzhalgay, Marituy, and Shumika, the port of Baikal and the estuary of the River Alanka can be referred to this group.

Collection of seed. About 30 g of seed were sampled from populations in various ecological communities within the territory of the Baikal–Lena's State Reserve: in the capes of Ryty, Sagan-Moryan, (3–4 m higher than the level of Baikal) and Pokoyniki (two populations, 800 and 1200 m above sea level), and in Solntsepad. Seed samples were taken as well as from populations in diverse areas of the Near-Baikal State Natural National Park.

Creation of plantations *in situ*. Plantations of reintroduced populations of 500-1500 seeds were created in the middle of September in sites at various elevations (460, 800 and 1250 m above sea level) in the Baikal–Lena's State Reserve in the cape of Pokoyniki, in Solntsepad, and in the Zavorotnaya Bay. Plantations were placed in glades of various grass - larch forests in the original habitat of plants. The site was dug, removed of roots and turf, and then layered with imported meadow soil mixed with sand. A mother plantation (total area of 4.68 m^2, located 15 m from the lake shore) was created in the territory of the Baikal–Lena's State Reserve, close to the base of the specially protected territory in a private vegetable garden of a staff member (Fig. 11). First, the site was dug; then, the upper layer of soil was mixed with sand and small pebbles. The soil represented light clay with small pebbles and sand and a humus content of 4.0–5.5%.

The surface was leveled by rakes. The planting scheme consisted of rows about 5 mm in depth with an inter-row span of 10 cm. Plantations were fenced and labeled with information boards. In the territory of the Near-Baikal State Natural National Park, the sites used for planting of mature seeds were adjacent to the cores of two pattern populations ("Uzur" and "Idiba", on the east bank of Olkhon Island), in order to expand the habitat areas of these populations and keep them in their relatively optimal state.

Plantations *ex situ*. *A. altaicum* has been grown in the Botanic Garden of Irkutsk State University since 1976. Plants develop normally, blossom and fruit annually, are self-sowing and grow vegetatively. The main introduction plantation for cultivation and production of seeds from the Baikal–Lena's State Reserve and the Near-Baikal State Natural National Park was created in the Botanic garden of Irkutsk State University within a territory of about 200 m^2 that was fertilized with about 800 kg of humus and 400 kg of perlite. The site was ploughed up by mini-cultivator and divided into beds. Seeds of four populations of *A. altaicum* were sowed in a greenhouse. Sprouts emerged in 5 days. Then, the *A. altaicum* seedlings were placed in special cavities (1500 specimens). In the middle of May, seedlings were transplanted into the prepared site. Besides transplanting seedlings, seeds were also directly sowed and they showed better quality (total amount of about 2500 specimens). Thus, the technology of transfer and return of the genetic pool from seeds grown in the Botanic garden of Irkutsk State University was used later for transportation and planting *in situ* on the west bank of Lake Baikal. In the summer, plantings were regularly tended (watering, weeding) and monitored: morphometric parameters were measured, and phenological observations were conducted. The staff from both participating specially protected areas was trained.

Monitoring. Plant development *in situ* and *ex situ* was monitored, if possible, once every 5 days. Staff of specially protected territories counted living plants, and measured morphometric characteristics of model specimens, in order to study the dynamics of their development [Stepantsova, 2006]. The first sprouts emerged on June, 15; the last ones on August, 16–21. Due to prolonged germination, a large variation in the habit of 1-year-old plants was observed. Further monitoring of the shores of Lake Baikal was implemented by huntsmen and staff botanists of the specially protected territories according to a special guidance by A.E. Turuta, entitled "Methodological recommendations for forest protection staff of the Near-Baikal Park for the reintroduction of populations of *A. altaicum*" [Turuta, 2003; Turuta et al., 2003]. Reintroduction was successful (Fig. 12) and accepted by a Special State Commission in 2003 and the Irkuts Local Authority Commission, which checked activities of the specially protected areas in 2003–2004. The project was supported with a great amount of information by local media, and work with locals and tourists was also organized.

Conclusion. As a result of the reintroduction of *A. altaicum*, a general scheme was formed showing the position that a botanic garden can play within the framework of specially protected natural landscapes and the circulation of plants for introduction, preservation and reintroduction of damaged populations [Kuzevanov, Sizykh, 2005; Kuzevanov, Sizykh, 2006] (Fig. 13).

4.4. Reintroduction of *Primula veris* in the Vladimir Region

Author: R.Z. Saodatova

Primula veris L. *(Primulaceae)* is a European species with a large ecological range. It can be encountered in meadows, slopes and glades, and open forests in all districts of Vladimir Region. It reproduces with seeds and propagates vegetatively. The number of populations of *P. veris* has been curbed due to picking of flowers and plowing of meadows; this is why this species is included in the regional list of protected plants.

From 2000–2003, reintroduction of *P. veris* was conducted in the Vladimir Region in a forest-park area of the city of Kirzhach.

One of the criteria for the creation of artificial populations of plants is the presence of a mass of original material. Therefore, a nursery for growing seed material was created. Mother plants of *P. veris* (Fig. 14) were grown from seeds of a 1999 introduction in the Botanic garden of Tver State University.

Specimens of *P. veris* in the nursery are characterized by speedy ontogenesis compared to their ontogenesis *in situ*. During the first year, they reach the immature state, the next year they reach the non-flowering state, and the third year enter generative period. *P. veris* has robust self-sowing in the nursery.

V.P. Belkov et al. [1993] showed that *P. veris in situ* can be successfully reproduced by division of a mother plant into daughter rosettes. The mother specimen can be divided in the spring while blossoming into 5-13 daughter rosettes. This method helped to get material in the nursery for planting an artificial population *in situ*. Planting material should vary in age, and include mature plants and younger plants grown from locally reproduced seeds. Locally reproduced seeds were collected when they were mature; then, they were planted in plots of the nursery.

For determining sites for reintroduction of *P. veris,* an ecological and plant community analysis of 40 forest communities using the scale of D.N. Tsyganov [1983] was performed. Eventually, pine and fir forests with cereals and various grasses were selected that were located in 23 blocks of Kirzhach forestry.

Planting of *P. veris* was made in the spring and summer in groups containing 16-30 specimens. The total number of specimens planted in the forest was 93, including 63 mature plants obtained by vegetative reproduction and 30 juvenile plants grown in the nursery from seeds locally reproduced in 2002. Mature plants were transplanted 15–20 cm away from each other in 10x10 cm wide, 12 cm deep holes (Fig. 15); juvenile plants, at a distance of 7 cm from each other in holes 3x3 cm wide and 4 cm deep (Fig. 16). Planting was performed with a minimum of damage to the soil cover. Before making holes, the surface moss cover was carefully removed. A hole with a plant was filled with nursery soil together with forest soil. After planting, soil was slightly compressed, watered and covered with moss. In summer, in dry weather plants were watered.

Establishment of mature plants by autumn comprised 96,8% or 61 plants; of 17 juvenile plants (56,7%), 5 remained in the same age state and 12 reached the immature state. Artificial population specimens have a high level of inner-population variability as indicated by the majority of morphological features.

The artificially created population of *P. veris* is heteromorphic and contains different aged speciments, allowing the preservation of this species in the forest park zone of the city of Kirzhach and further monitoring of its condition.

APPENDIX

RULES FOR THE COLLECTION OF RARE AND ENDANGERED SPECIES OF PLANTS (for botanic gardens) [Pravila sbora..., 1981, updated]

Human-generated changes in the biosphere have led to a reduction in the abundance and spread of many wild growing plants and put species under the threat of extinction. All the possible methods and tools to prevent impoverishment of the flora from progressing must be used.

No doubt that survival of rare and endangered species, first and foremost, depends on protection of habitats. However, cultivation of target plant species in botanic gardens can provide additional guarantee of their survival.

Therefore, in recent years Russian botanic gardens have been actively developing efforts in this direction. These efforts include considerably expanding the collection of rare and endangered species *in situ*, especially in cases where not to follow necessary protective measures would result in a reduction of the threatened species natural populations. In order to regulate these activities the following rules regarding the collection of rare and endangered species have been worked out. This set of rules concerns the activities of botanic gardens and is advisory.

1. All work on placing rare and endangered species *ex situ* by botanic gardens is targeted at their preservation, reproduction and research. These efforts should be planned and arranged in order to follow the framework of the jointly agreed activities of Russian botanic gardens. Botanic gardens ought to concur in advance their plans on introduction and reintroduction of rare species with the Commission on Rare Species of Plants at the Russian Botanic Garden Council and the regional divisions of the Russian Botanic Garden Council, as well as to inform these bodies about the process and results of these works regularly and in a timely manner.

2. When planning for the introduction of rare species and before the collection of natural material, a botanic garden should evaluate its actual capacities for providing favorable conditions that meet the demands of the given species, its growth and its sustainable management over quite a long period (at least for 1–2 decades in the future).

3. When planning for the introduction of rare and endangered species as well as at their collection *in situ*, botanic garden staff must be aware and follow all environmental laws and regulations (both federal and regional). Garden staff should use all opportunities to explain environmental protection measures to the public, as well as to grassroots and state organizations. Before the beginning of a field expedition, staff should learn the existing lists of rare and endangered plants growing in the area of interest. They should also know which conservation territories and wildlife preserves are located in the given area. In wildlife preserves all types of field work can be implemented only with permission, given to experts in appropriate instances.

4. Any collection of *in situ* live plants or seeds of rare plants should be conducted only after sufficient study of the condition of the population and the extent to which the collection would be detrimental to the existing population. Thus, even one-time collections should be prepared by an investigation of the population. Repeated collection of material from a population of rare plants is allowed only if a botanic garden can commit to regularly monitoring the condition of the population.

5. If possible, in the course of field work, the botanic garden staff should examine the condition of those rare species populations encountered, for which cultivation is not planned within the garden. It is worth examining, in case unusual habitats of rare species are found that have not been known before.

6. Besides word description, each examination of rare or endangered species population should be accompanied by schematic depiction of location in plan (for possible repeated finding), by pictures of individual plants and the whole habitat, and by collection of herbarium samples (if this collection is allowed given the population number). Herbarium samples are submitted to herbaria which provide sustainable and satisfactory conditions for very long storage of samples.

7. Collecting material *in situ*, one should account for the category of the general condition of the species. In any case, it is preferable to collect seeds or cuttings and it is better to avoid taking whole live plants, in particular, mature ones. Introduction of species categories 0 or 1 is indicated only by picking seeds or cuttings and only for preservation, as agreed with the Commission on Rare Species of Plants at the Russian Botanic Garden Council; their collection for scientific purposes is absolutely prohibited.

8. Working *in situ* one should be very careful with habitats of rare and endangered plants; do not break or trample down the surrounding plants, leave holes, attract the attention of dangerous people, or disseminate information concerning habitats among non-specialists.

9. Complete removal of a rare species population (including populations which account for only 2–3 plants) is recommended only in cases of immediate and complete elimination of the whole habitat.

10. Collection, transfer, planting and care of rare plants in botanic gardens must be implemented only by experienced staff or at least, guided by day-to-day high-profile supervision. Special attention is paid to the detailed documentation of collected samples, the maximum full ecological characteristics of plants, as well as the correct preparation of materials for transport (taking into account the ecology and live forms of the plants) and quick delivery to the destination.

11. In order to increase success of rare plant cultivation, botanic gardens may share collected or reproduced material with other interested gardens. If introduction turned out to be successful, gardens will tend to endorse *ex situ* cultivation to as large extent as possible.

12. The highly important task for botanic gardens is the reintroduction *in situ* of rare species whose natural populations have completely or partly disappeared. All work on reintroduction must be well documented and covered in a special press.

13. Working out plans to introduce and increase the attraction of rare and endangered species, botanic gardens are willing to cooperate with other scientific establishments, the public, administrative bodies and, first and foremost, with other gardens. Councils encourage the organization of exchange of information between botanic gardens working in the field of preservation of rare and endangered species *ex situ*. When organizing expeditions and other works regarding introduction and reintroduction of rare and endangered species, botanic gardens should establish contacts with local environmental organizations.

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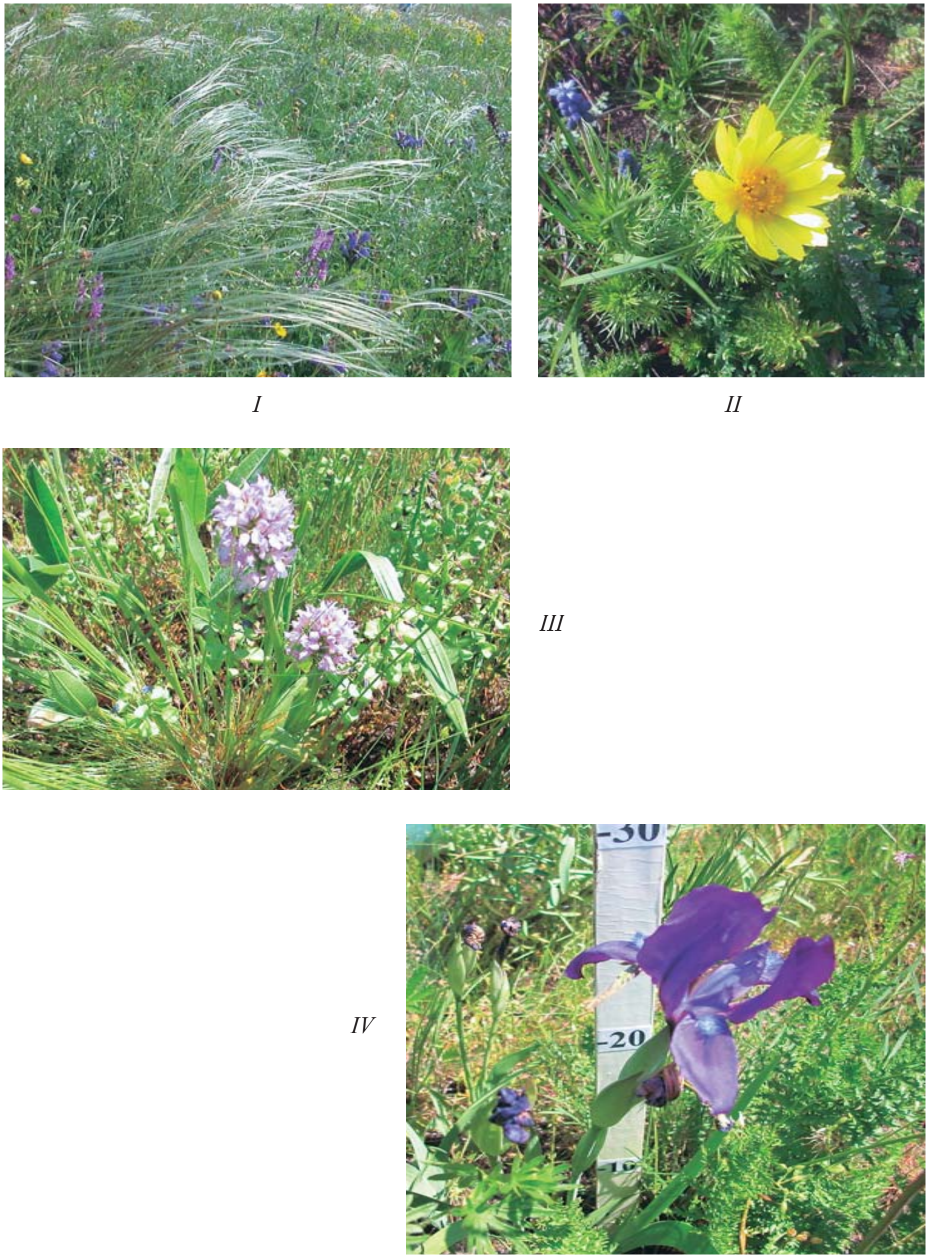

Fig. 1. I. — *Rich various grass, turf and cereal 27-year old steppe with rare species of plants (Stipa pennata L., S. pulcherrima C. Koch, Paeonia tenuifolia, Anemone sylvestris, Crocus reticulates and others), 20 km to the North of Stavropol; II — Adonis vernalis L.; III — Orchis tridentata Scop.; IV — Iris aphylla L. (photos by D.S. Dzybov)*

Fig. 2. *Appearance of Rhodiola iremelica A.Boriss. (flowering) (photo by L.M. Abramova)*

Fig. 4. *Rentroduction nursery for the low-mountainous (shade) form of Rhodiola iremelica A. Boriss. (photo by L.M. Abramova)*

Fig. 3. *Habitat of the low-mountainous (shade) form of Rhodiola iremelica A. Boriss. (photo by A.A. Muldashev)*

Fig. 5. *Reintroduction material of Rhodiola iremelica A. Boriss., grown in the nursery (photo by L.M. Abramova)*

Fig. 6. *1-year-old planted specimen of Rhodiola iremelica A. Boriss. (photo by L.M. Abramova)*

Fig. 8. *Planting of Allium hymenorhizum Ledeb. in situ (photo by A.A. Muldashev)*

Fig. *7. Fruiting plant of Rhodiola iremelica A. Boriss., 2 years after planting (photo by A.A. Muldashev)*

Fig. 9. *Allium hymenorhizum Ledeb., 2 years after planting (photo by A.A. Muldashev)*

Fig. 10 *Habitat distribution of Allium altaicum Pall. according to the Red Book of the Russian Federation*

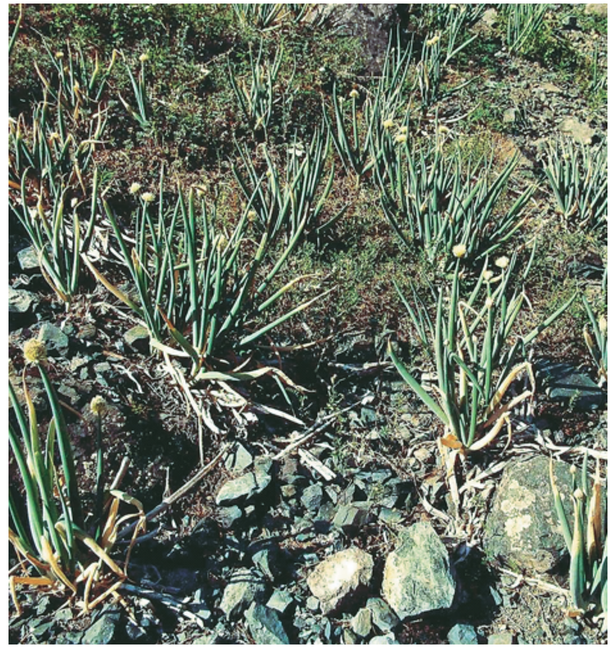

Fig. 12. *Reintroduced population of Allium altaicum Pall. at the west bank of Lake Baikal (photo by N.V. Stepantsova)*

Fig. 11. *Mother plantation of Allium altaicum Pall. in a flower bed close to the base of the Baikal – Lena's State Reserve in the west bank of Lake Baikal (photo by N.V. Stepantsova)*

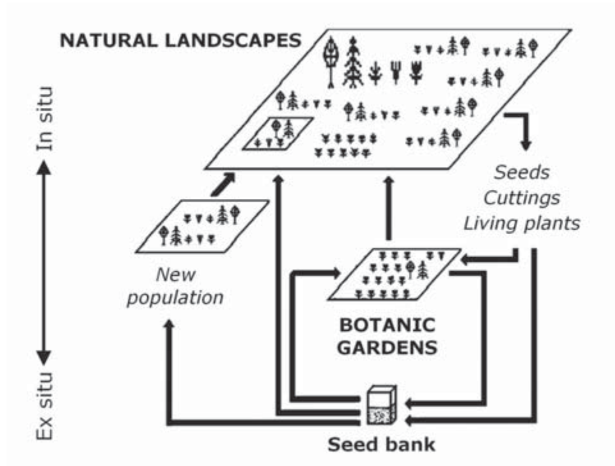

Fig. 13. *Position of Botanic Gardens in a system of plant genetic resources circulation for introduction, in situ and conservation ex situ, as well a for the restoration of damaged populations [Kuzevanov, Sizykh, 2005, 2006]*

Fig. 14. *Mother plants of Primula veris in the nursery (photo by R.Z. Saodatova)*

Fig. 15. *Mature plants of Primula veris in reintroduction experiment (photo by R.Z. Saodatova)*

Fig. 16. *Juvenile plants of Primula veris in reintroduction experiment (photo by R.Z. Saodatova)*