Intensive Care for Critically Endangered Plants

*** What this guide is about, and why.**

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*	***Step 2***	**How much, what and when to collect?**
*	***Step 3***	**How to maintain the species *ex situ*?**
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C ESA Policy Regarding Controlled Propagation of Listed Species.
D Guidelines to Reduce Impacts of Hybridisation.
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What this guide is about, and why.

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What this guide is about, and why.

Many plant species are reduced to very dangerously low population levels.

According to the IUCN[i] Red List of Threatened Species™, in 2004 no fewer than 3,729 plant species were evaluated as endangered (facing a very high risk of extinction in the wild). Of these 1,490 were *critically* endangered, i.e. facing an *extremely high risk*.

These figures are not only increasing (2004 figures are 29% higher than 2003) but are likely to be a significant underestimate: only a tiny percentage of all described plant species were included in IUCN's evaluations. Occasional rediscoveries of species previously considered extinct also present situations where maybe no more than a handful of individuals remain.

Reintroduction of plant species to the wild will become an increasingly important conservation and management tool and this work sets out to develop a stand-alone, pragmatic guide for the use of horticulture as a conservation tool in such situations, suitable for local use in all regions.

Such a one-size-fits-all solution cannot be perfect, but it is hoped that through evolution and local adaptation it can contribute to the conservation of the world's most endangered plants. This guide is being 'launched' along with discussions about how it should develop, through the Alliance for Sustainable Horticulture, www.sustainablehorticulture.org

It is also hoped that this guide will make a contribution to achieving the targets of the Convention on Biological Diversity's Global Strategy for Plant Conservation and the 2010 Targets for Botanic Gardens that include the ambitious commitment to help conserve 75% of the world's critically endangered plant species by 2010.

Context and Approach

In general the areas of the world with the greatest need for *ex situ* plant conservation facilities have the least resources[ii]. But not only are resources short, often there are large gaps in knowledge about horticulture, genetics, population dynamics and viability for critically endangered species. Moreover, while in recent years there have been advances in 'high-tech' approaches such as seed, pollen, tissue and germplasm management, relatively little attention has been focused on more basic horticultural questions and needs.

Although *ex situ* recovery programmes have been shown to be difficult, complex and often expensive, this work aims to:

* **focus on the achievable and immediate,**
* **take account of real world situations where time and resources are short,**
* **acknowledge that acute conservation needs may outweigh delays that would be incurred by extensive research and planning.**

Scope of the Guide

This guide focuses in turn on six major questions that it will be necessary to answer for critically endangered plants.

Step 1 **Is this a species that needs emergency horticulture?**

Step 2 **How much, what and when to collect?**

Step 3 **How to maintain the species *ex situ*?**

Step 4 **How to move towards a successful reintroduction?**

Step 5 **What to record, measure and monitor?**

Step 6 **Where to turn for help and information?**

Wherever possible this guide will attempt by means of 'decision trees' and summaries of key factors to lead the field practitioner through the many interwoven and complex issues to wise answers. All reintroduction initiatives will benefit from a detailed Reintroduction Plan that considers objectives, methods, scale, schedule, responsibilities, resources and criteria to measure success. This guide should help in the development of such a plan.

Correct decisions require good information, maybe about seed viability, the availability of natural pollinators, or the genetic distinctiveness of different remnant populations. Yet in many cases not all the information will be available, and if extinction seems imminent there may not be time to collect it. In most cases a difficult judgement will have to be made about what information it is appropriate or timely to collect before taking action, but researching a species to extinction is not the intent of this guide.

Those making decisions on the ground are strongly urged to record their decisions, efforts, successes and failures, and to share them, to help others make good decisions in the future. What to record and monitor is summarised in ***Step 5*** but it is also important to consider how success will be judged.

While all projects may have the goal of creating self-sustaining wild populations, every project will benefit from more specific goals that will allow the success – or otherwise – of any reintroduction to be re-evaluated. These will likely centre around four key attributes:

* abundance,
* extent,
* persistence over time, and
* resilience (i.e. ability to withstand and recover from environmental variations).

Setting goals for these prior to reintroduction is an important part of the process, and the reader is also recommended to consult Pavlik (1996)[iii].

The answer to many questions in the decision trees may well be 'don't know'. In some cases guidance is given as to how to act in the absence of a clear answer, but in others the conservationist, botanist or hortulturist on the ground may have to make difficult decisions. ***Step 6*** - Where to Turn for More Information - is thus an important part of this guide.

A Word about Words

For the purposes of this work:

* A 'critically endangered plant ' is one judged to be facing an extremely high risk of extinction in the wild and for which urgent intervention is required to mitigate threats to its survival.

* 'Horticulture' is human-induced propagation of plants from seeds, spores, callus tissue, divisions, cuttings, or other plant tissue, or through pollination in a controlled environment[iv]'.

While this guide refers to 'species' it could equally, where appropriate, be applied to any subspecific taxa.

And while this guide generally refers to 'reintroduction' in most cases it could equally be applied to introduction, augmentation or translocation of plants or populations.

In situ and ***ex situ***

Even if measures are in place to address threats to a species in the wild there can be a role for horticulture to ensure sufficient individuals are available for future reintroduction, to optimise retention of genetic diversity, and of course to provide an insurance policy should the species become extinct in the wild. Indeed a wise combination of *in situ* and *ex situ* approaches will be necessary for many, if not most critically endangered plants.

This guide assumes that parallel initiatives are underway to research the nature of threats to the species, and where possible to pursue options for *in situ* population recovery. This guide should thus be seen as providing a *component* of a recovery strategy for a critically endangered species.

This guide also takes the view that the combination of *in situ* conservation with a local *ex situ* horticultural approach offers a number of potential benefits, such as those below:

* Access to local knowledge, support and expertise.
* Local development of that knowledge, support and expertise.
* Often providing a more straightforward access to appropriate climatic and horticultural conditions.
* Providing access to propagation material over an extended period.
* Ensuring a close focus is retained on research and development of the local measures and conditions necessary for long-term *in situ* conservation, and thereby avoiding the danger of *ex situ* conservation resulting in 'museum species'.

There are of course questions about the balance of allocation of limited resources between habitat conservation on the one hand and preservation of species on the brink of extinction on the other. These balancing acts can only be resolved at local level and are beyond the scope of this guide.

The IUCN guidelines for management of *ex situ* populations for conservation should be followed. Refer to **Appendix E**.

Teamwork

This guide assumes at all stages that work will be carried out in consultation and co-operation with landowners, scientific, cultural and legal authorities, and with respect for local knowledge, traditions and sensitivities.

Development of this guide continues to be informed by many initiatives and publications from around the world and the hard work of many dedicated workers. It is hoped their contributions are adequately represented. All feedback and suggestions are welcome.

Step 1

Is this a species that needs emergency horticulture?

1.1 Key preliminary questions

1.2 Is the species critically endangered?

1.1 Key preliminary questions

Every collection from an endangered wild population contributes, however slightly, to the risk of extinction. As will be seen in ***Step 2*** ex situ populations can also contribute directly and indirectly to the threats to a species. Therefore, even when great concern is felt about the fate of a species in the wild, a series of questions needs to be answered to determine whether a species is a suitable candidate for conservation horticulture.

1. Are you sure about the taxonomy?

Yes/No

Is it an undisputed, formally described species? Is it part of a species complex that requires further study? Hybrids, subspecies and variants way not warrant the same conservation priority as distinct species.

2. Are initiatives underway to understand and where possible address threats to wild populations?

Yes/No

3. Have temporary *in situ* measures been adequately considered (and judged inadequately successful)?

Yes/No

Temporary *in situ* measures should be considered while more sustainable, longer-term solutions are developed. These could include removal or exclusion of herbivores (large or small), weeding, watering, shading, etc. Even small scale *in situ* management (such as removing competing plants or protecting from herbivores by wire cages) can be effective in buying time while more sustainable solutions are found, and can avoid the many challenges and risks of management and reintroduction of *ex situ* populations. If the species is endangered because of missing 'mutual' species (e.g. pollinators or seed dispersers) or ecological processes (e.g. fire or grazing), artificial pollination or seed dispersal or controlled burning or grazing could be carefully attempted.

4. Is enough known of the species' horticultural requirements to ensure an *ex situ* collection can be maintained?

Yes/No

5. Do sufficient facilities and resources exist to ensure that an *ex situ* initiative has a reasonable prospect of maintaining and reintroducing the species as described in *Step 3* and *Step 4*?

Yes/No

Are these facilities and resources sufficiently viable over the long-term to provide adequate security for a valuable *ex situ* population?

6. Has legal permission been obtained, if necessary, to collect material?

Yes/No

7. Are efforts underway to consult with interest groups, landowners, botanical and horticultural experts?

Yes/No

If the answer to all the questions above is yes, it is still necessary to carefully determine that the species is *sufficiently endangered* to warrant moving on to ***Step 2***.

1.2 Is the species critically endangered?

How to determine whether a species is critically endangered has been much debated and resulted in IUCN's Criteria for Endangered and Critically Endangered Taxa. The full, complex definitions and criteria for 'endangered' and 'critically endangered' are given in Appendix A. This decision key is based on the IUCN criteria.

A1 Is the population 50 or fewer mature individuals? Go to ***Step 2***.
More than 50 mature individuals? Go to **A2**.

A2 Is the population 250 or fewer mature individuals? Go to **A3**.
More than 250 mature individuals? Go to **A7**

A3 Is the population in continued decline by 25% over 3 years or one generation (up to a maximum 100 years) whichever is longer? Go to ***Step 2***.
Population in decline though not as above? Go to **A4**.
Population not in decline? Go to **A7**.

A4 Is there extreme fluctuation in the number of individuals? Go to ***Step 2***.
Fluctuation in individuals not extreme? Go to **A5**

A5 Are at least 90% of mature individuals in one subpopulation? Go to ***Step 2***.
Fewer than 90% of mature individuals in any one sub-population? Go to **A6**.

A6 Does at least one population have more than 50 individuals? Go to **A7.**
No population estimated to have more than 50 mature individuals? Go to ***Step 2***.

A7 Is the area of occupancy less than 10km^2? Go to **A8**.
Area of occupancy more than 10km^2? Go to **A13**.

A8 Is the population severely fragmented or known from only a single location? Go to **A9**.
Population neither severely fragmented nor known from only a single location? Go to **A11**.

A9 Is there continuing decline[v] in any of: extent or area of the species, area, extent or quality of habitat, number of

> **Area of occupancy** is the "smallest area essential at any stage to the survival of existing populations".
> **Extent of occurrence** is "the area contained within the shortest continuous imaginary boundary which...encompasses all the known, inferred or projected sites of present occurrence of a species, excluding cases of vagrancy".

locations or subpopulations, or number of mature individuals? Go to ***Step 2*****.**
Not a continuing decline in any of the above? Go to **A10**.

A10 Are there extreme fluctuations in any of: extent or area of the species, number of locations or subpopulations, or number of mature individuals? Go to ***Step 2***.
Not extreme fluctuations in any of the above? Go to **A13**.

A11 Is there continuing decline[v] in any of: extent or area of the species, area, extent or quality of habitat, number of locations or subpopulations, or number of mature individuals? Go to **A12**.
Not a continuing decline in any of the above? Go to **A13**.

A12 Are there extreme fluctuations in any of: extent or area of the species, number of locations or subpopulations, or number of mature individuals? Go to ***Step 2***.
Not extreme fluctuations in any of the above? Go to **A13**.

A13 Is the extent of occurrence less than 100km^2? Go to **A14**.
Extent of occurrence more than 100km^2? Go to **A19**.

A14 Is the population severely fragmented or only known from a single location? Go to **A15**.
Population neither severely fragmented nor only known from a single location? Go to **A17**.

A15 Is there continuing decline[v] in any of: extent or area of the species, area, extent or quality of habitat, number of locations or subpopulations, or number of mature individuals? Go to ***Step 2***.
Not a continuing decline in any of the above? Go to **A19.**

A16 Are there extreme fluctuations in any of: extent or area of the species, number of locations or subpopulations, or number of mature individuals? Go to ***Step 2***.
Not extreme fluctuations in any of the above? Go to **A17**.

A17 Is there continuing decline[v] in any of: extent or area of the species, area, extent or quality of habitat, number of locations or subpopulations, or number of mature individuals? Go to **A18**.
Not a continuing decline in any of the above? Go to **A19**.

A18 Are there extreme fluctuations in any of: extent or area of the species, number of locations or subpopulations, or number of mature individuals? Go to ***Step 2***.
Not extreme fluctuations in any of the above? Go to **A19**.

A19 Are the causes of reduced population size clearly reversible and understood and ceased? **Go to A20**.
Causes of reduced population size not as above? Go to **A21**.

A20 Has the population reduced by 90%[vi] or more over the last 10 years or three generations, whichever is the longer? Go to **Step 2**.
Population not reduced by 90% or more as above? Go to **A21**.

A21 Has the population reduced - or is it expected to reduce - by 80%[vi] or more over a 10 year period (or three generations if longer) that includes the present day? Go to ***Step 2***.

Population not reduced or expected to reduce as above? Go to **A22**.

A22 Has quantitative analysis showed a probability of extinction in the wild of at least 50% within 10 years or three generations, whichever is the longer (up to a maximum of 100 years). Go to **Step 2**.
Quantitative analysis has not showed a probability of extinction as above? The species does not meet IUCN's criteria for 'critically endangered'. Consider other courses of action that enhance the survival prospects for the species' survival in the wild.

Too many species meet the criteria for the resources available?

Where there are more critically endangered species requiring emergency horticulture than resources allow, other criteria may have to be used to determine which to prioritise while further resources are sought. These include whether a species has particular ecological value to the long-term survival of its habitat, or whether it has economic, medicinal, cultural or emblematic value.

The species is critically endangered, initiatives (where possible) are underway to address the threats to wild populations, in situ *horticultural options have been explored and if underway are still inadequate, legal permission has or will be obtained to collect material, consultation is underway with interest groups and experts, secure facilities and resources exist to maintain an* ex situ *population. A series of decisions is now necessary to determine how much, what, and when to collect from wild populations.*

Step 2

How much, what and when to collect?

2.1 How much?

Emergency measures
Minimising impacts on remaining wild populations.
Ensuring sufficient material will be available for reintroduction.

2.2 What to collect?

What parts or life-stages?
Which individuals?
More than just the plant?

2.3 When and how often to collect?

2.4 Collecting techniques

2.1 How much?

Emergency measures

Experience from the animal conservation shows occasional examples of species in catastrophic decline where all remaining individuals have been removed from the wild, prior to a successful reintroduction. But this can be a very risky strategy and is best avoided.

The following emergency measures are suggested when there are 50 or fewer reproductive individuals believed to remain in the wild.

- 50 or fewer reproductive individuals -

B1 Are 10 or fewer reproductive individuals believed to remain? Go to **B2**.
More than 10 reproductive individuals believed to remain? Go to **B7**.

B2 Does the species have either a poor history of recruitment or is it known to be in rapid decline? Go to **B3**.
Species neither with poor history of recruitment nor known to be in rapid decline? Consider issues raised in ***Step 2*** and go to **C1**.

B3 Is viable wild-set seed available? Collect 100% of seed. Go to ***Step 3***.
Viable wild-set seed not available? Go to **B4**.

Guidance on assessing seed viability is given in ***Step 3***.

B4 Are remaining individuals in imminent threat? Go to **B5**.
Remaining individuals not in imminent threat? Go to **B6**.

B5 * If remaining individuals are in imminent threat from *unstoppable habitat destruction*, take plant parts for propagation and translocate entire plants. Go to ***Step 3***.
* If remaining individuals are in imminent threat from *herbivores*, take measures to remove or exclude these herbivores. Go to **B6**.
* If remaining individuals are in imminent threat from *extreme climatic conditions* take measures to ameliorate these threats (e.g. watering, shading). Go to **B6**.

B6 Take whatever measures are possible to increase the wild population, and judiciously take plant parts (cuttings, etc.) *ex situ*. Go to ***Step 3***.

B7 Are 50 or fewer reproductive individuals believed to remain? Go to **B8**.
More than 50 reproductive individuals believed to remain? Consider issues raised in ***Step 2*** and go to **C1**.

B8 Does the species have either a poor history of recruitment or is it known to be in rapid decline? Go to **B9**.
Species neither with poor history of recruitment nor known to be in rapid decline? Consider issues raised in ***Step 2*** and go to **C1**.

B9 Is viable wild-set seed available? Collect seeds judiciously from *all* seed-bearing plants (perhaps no more than 20% of all available seed unless the population is threatened with immediate destruction) and maintain every genetic individual through vegetative propagation (for subsequent seed production and/or reintroduction). If necessary make carefully planned crosses *ex situ*. Go to ***Step 3***.
Wild-set seed not available? Go to **B10**.

B10 * If any remaining individuals are in imminent threat from *unstoppable habitat destruction*, take plant parts from these individuals for propagation and translocate entire plants. Go to ***Step 3***.
* If remaining individuals are in imminent threat from *herbivores*, take measures to remove or exclude these herbivores. Go to **B6**.
* If remaining individuals are in imminent threat from *extreme climatic conditions* take measures to ameliorate these threats (e.g. watering, shading). And follow **B6**.

Except in the emergency situations described above, the decision on how much to collect centres on two requirements:

A. *Avoiding or minimising impacts on remaining wild populations.*
B. *Ensuring sufficient and appropriate material will subsequently be available for reintroduction.*

A. Avoiding or minimising impacts on remaining wild populations.

In most cases the sources of endangered plants are extremely limited and may be placed at further risk by over-collecting. Except in salvage operations, any collecting from the wild increases, however slightly, the risk of extinction.

Seed can generally be collected in greater numbers than cuttings or whole plants, and generally with lesser impact. Seed removal may nonetheless reduce the population growth rate and increase probabilities of extinction, especially for annual species without persistent seed banks.

Frequent low-intensity harvests are better than infrequent high-intensity. While it varies from species to species, as a general rule harvesting 10% of seeds no more than once every 10 years does not increase extinction risks[vii]. Harvesting more than 50% of seeds more than every two years does pose a significant risk, unless the population is over 500. In terms of impacts on individual plants, FloraBank suggest no more than 20% of seed from any one plant and no more than 10% of plant material from any plant[viii].

B. Ensuring sufficient and appropriate material will be available for reintroduction.

Generally samples should attempt to capture at least 95% of the genetic variation, and a minimum of 500 samples has been suggested to achieve this. For some critically endangered plant species this may of course be a hopelessly high target.

The need to minimise risks to wild populations will mean that the maximum number that can be collected - even over a number of years - may be less than 'RN' - the numbers of individuals required for a successful reintroduction. If so then collect the maximum possible bearing these risks in mind, and then maintain and increase numbers in cultivation as described in ***Step 3***.

RN = Number required *ex situ* to allow for successful reintroduction

The major objective of the ex situ population here is to provide sufficient, suitable stock at a time when a suitable reintroduction site is available. It may seem odd to consider RN before deciding what material to collect from the wild. However as will be seen in **2.2** below, assessment of RN can in some situations influence what to collect. Given the many factors involved and the likelihood of gaps in knowledge, assessing RN is unlikely to be an exact science.

In many situations RN will be *greater* than the minimum viable wild population as in general the larger the 'founding population' the greater will be the chance of its survival to a self-sustaining position. While there is a strong link between the two, RN is not the same as the Minimum Viable Population (MVP).

In some situations, it may be reasonable to reintroduce a much smaller number, e.g. small numbers of sexually mature adults that might produce large amounts of viable seed. In any case the following practical and ecological considerations need to be borne in mind.

Assessing RN - Ecological Considerations

RN or MVP likely to be **Smaller**	RN or MVP likely to be **Larger**
Long lived/perennial	Short lived/annual
Largely out-crossing	Largely inbreeding[ix]
Historically rare	Recent population decline
Not especially susceptible to random environmental variation	Susceptibility to random environmental variation
Most individuals survive to reproduce	Few individuals survive to reproduce
Monoecious	Dioecious
Largely sexually reproducing	Largely clonally reproducing
Most frequent in climax habitat successional stage	Most frequent in an early successional stage
Prolific production of seed or other propagules	Seed or other propagules not produced prolifically
Healthy populations of pollinators and seed dispersers	Populations of pollinators and seed dispersers threatened

While it is very difficult to provide specific numerical advice, the aim should be to maintain a population of certainly *hundreds* and preferably *thousands* of genetically distinct individuals *ex situ*, the actual scale of the required population depending on factors including those listed in the table below. A species showing most of the characteristics in the left-hand column in the table above might have a MVP of 50; one showing most of the characteristics in the right-hand column might have a MVP of 2,500.

Assessing RN – Practical Considerations

* Equipment failures, human error, disease, and other potential catastrophic events may all cause the loss of some or all the *ex situ* population. Moreover, reintroduction is not always successful, either through inadequate understanding of the species' ecology and threats, or unexpected environmental or ecological extremes. A number of attempts may be required and if insufficient *ex situ* material is held in reserve not only may valuable stock be lost but also the species might be put through a genetic bottleneck with subsequent inbreeding depression.

* Losses are likely shortly after planting/sowing and may necessitate reintroduction of a much greater number than would comprise a viable wild population. Up to 98% losses have been recorded before a reintroduced population begins to recover, and the remnant population from such a bottleneck may have reduced long-term viability. Attempting to prevent losses *after* reintroduction might be an understandable desire but it should be borne in mind that doing so could well slow the necessary adaptive response of the new population to its environment. Losses are likely to be greater for species that have had to be maintained *ex situ* by sexual propagation, due to the likely domestication (see ***Step 3***) resulting from such processes.

* Material may be required for research on germination, propagation and, reintroduction.

* If seed storage is a chosen technique, remember that a dead seed looks much like a live seed, so regular assessment of viability may be required. Sample sizes required to assess declines in viability are remarkably large[x].

2.2 What to Collect?

This second decision centres on two issues:

A. What parts or life-stages of the species to collect?

B. Which individuals to collect from?

A. What parts or life-stages to collect?

A summary is presented here of the various options, followed by a decision tree.

Seed.

Generally the best option for three important reasons:

* Seed collection should have lesser impact on remaining wild populations,
* If sampled across the remaining population/s seed collected will likely retain the species' genetic diversity.
* If seed can be stored the resources required to maintain RN are relatively small.

Seed storage avoids the domestication that affects a collection of growing plants in an environment that is bound to be more or less different from natural conditions. Moreover pests and diseases are much less likely to be a problem for properly stored seeds than living plants. Likewise with spores for pteridophytes and bryophytes.

Keep seed from each maternal parent separate to make it possible to equalise the contribution of different parent plants in reintroduction.

The timing of seed collection needs careful considered. Ideally to maximise capture of genetic diversity it should be spread right across the times of year when seed is produced. However, delay can mean seed has been shed and becomes hard to collect. Consequently spread seed collection over as many occasions as possible.

Pollen.

Collecting and storing pollen has some advantages similar to seed collection and is worth considering[xi]. It also allows pollination when male and female flowers open at different times, or when wild pollinators have declined or are extinct. Additionally it allows controlled pollination between individuals, both wild and cultivated, and can thus increase effective population sizes, especially when there are geographically isolated individuals. Generally however pollen collection will be a useful adjunct to a seed or plant collection. After all there must in due course be something to pollinate!

Pollen is best collected as soon as possible after the anthers start to release pollen (often very soon after the flower bud has opened), ideally from healthy plants as immature and aged pollen and pollen from stressed plants can be poor quality. As quickly as possible, dry to 5-10% of fresh weight, pack and store in a freezer at low temperature.

Drying can be achieved over saturated calcium nitrate salt solution or saturated magnesium chloride solution. A freezer at -18C should extend pollen life to months or years. Optimum pollen collection and storage vary from species to species, so look for studies on closely related species as a guide. Rehydrating pollen requires placing at high humidity (e.g. over water) at room temperature.

Cuttings.

Where viable seed is not available, cuttings are the next option. Refer to any good horticultural textbook for advice on taking and rooting cuttings (e.g. the Darwin Technical Manual for Botanic Gardens[xii])

Sometimes cuttings from old or weak plants will themselves show low vigour. Where it is necessary to take cuttings from old or weak plants, try to take them from the most vigorous parts of the plant. If these cuttings root successfully consider boosting number by taking further cutting from these stock plants.

Moving such plants into tissue culture can re-invigorate the material. See ***Step 3***.

Any plant entering the collection is a potential source of disease. A quarantine system is advisable, as well as careful cleaning of pots and other equipment.

Bulbs, etc.

For species that produce bulbs or other storage or dormancy organs these can provide a very convenient means of moving plant parts.

Whole plants.

To be avoided unless crucial to the species' survival. Transplanting large, mature plants is a major operation, from which most plants recover slowly, if at all, and even in 'emergency' situations it should be carefully planned and carried out. Moving plants during a dormant season is highly recommended, otherwise try to choose a cool, still day. To allow the plant to become adequately hydrated, it should generally be watered 1-2 days prior to moving.

Minimising damage to the root system is critical, usually achieved by moving the plant with a large root-ball with soil undisturbed. The root ball should be watered again thoroughly prior to digging to keep the ball intact and reduce soil loss as much as possible during transport. As a general guide a plant with a 2.5cm diameter trunk needs a root-ball of at least 40cm diameter, a plant with a 7.5cm diameter trunk will need a root-ball of at least 80cm diameter. The depth of the root ball should be at least two-thirds of its width. If large roots have to be cut then use a sharp pair of loppers rather than a spade that will jar roots and loosen the soil around the roots.

Plants with root balls less than 60cm diameter are best moved by lifting from beneath with a piece of strong material which has been rolled underneath by tilting the ball first one way then the other. A plant should never be lifted from the hole by pulling on the above-ground parts. Plants with root balls larger than 60 cm diameter will generally require use of mechanical equipment. A 60cm root ball can weigh 150kg, a 90cm root ball 450kg.

No matter how carefully carried out damage will be done to the plants absorbing root area, and plants should be kept as free from water-stresses as possible until they have re-established. Plants being moved should be kept in the shade with the root ball covered with moistened wood chips, damp straw or equivalent. Particular care should be taken to avoid exposing roots to wind, the drying effects of which can kill a plant remarkably rapidly.

It is important when watering newly transplanted plants that the original soil ball and surrounding soil is saturated to a depth of 30cm. Apply water slowly to the entire area, allowing adequate penetration.

If a plant has suffered root damage and has had to be moved while in leaf it may be worth considering judicial pruning of some of the above-ground plants to reduce transpiration stresses. An alternative is to spray foliage with an anti-transpirant prior to transplanting.

Plants should not be transplanted to open, exposed locations. They may be structurally weak in addition to being particularly susceptible to water stresses.

A combination.

In many cases it could be wise to consider a combination of these options, to fulfil different needs of the *ex situ* maintenance and reintroduction process, and to act as an insurance policy.

More than just the plant?

The specific soil type for a species may be key to its survival – and success in cultivation. It is very much worth considering whether to collect sufficient soil from the collection site to use as a substrate to maintain the species *ex situ*, wherever this can be achieved without significant habitat damage. For some species the presence of the correct root-nodule forming bacteria (rhizobia) may be crucial to their successful cultivation and propagation. Beware, however, the risk of spreading soil-borne pathogens such as *Phytophthera*.

Where collecting plants or underground plant parts (rather than seed or cuttings), it is important to move plants with their soil and with roots undamaged so far as possible.

Tagging and labelling.

Tagging plants or plant parts or labelling fruit and seed needs to be done securely and immediately.

Throughout the following decision key it is assumed that due note will be taken of:
* precautions required to minimise damage to wild populations, and
* guidance on when and how often to collect.

C1 Is viable wild-set seed available? Go to **C2**.
Viable wild-set seed not available? Go to **C12**.

C2 If seed capable of storage? Go to **C3**.
Seed not capable of storage? Go to **C8**.

More information on assessing seed viability is given in ***Step 3***.

C3 Do facilities exist for storage and/or maintenance of RN? Go to **C4**.
Insufficient facilities for storage and/or maintenance of RN? Go to **C7**.

C4 Is sufficient wild-set seed available over a number of years[xiii] to achieve RN? Go to **C5**.
Insufficient wild-set seed available even over a number of years to achieve RN? Go to **C6**.

C5 Collect and store the number required for reintroduction.

C6 Supplement seed collection and storage with sufficient vegetative material[xiv], to allow a population increase up to RN in cultivation through vegetative propagation.

C7 Collect the maximum seed stock possible for which facilities exist, and supplement by the maximum vegetative stock for which facilities exist. Endeavour to obtain additional resources/facilities that permit RN to be reached.

C8 Is sufficient wild-set seed available over a number of years to achieve RN? Go to **C9**.
Insufficient wild-set seed available even over a number of years to achieve RN? Go to **C11**.

C9 Is reintroduction a realistic possibility within the reproductive life of germinated plants? Go to **C10**.
Reintroduction not a realistic possibility within the reproductive life of Germinated plants? Go to **C11**.

Ex situ seed-based propagation poses considerable risks of maladaptation through inadvertent artificial selection. Thus if the species will require seed–based propagation to maintain or increase the *ex situ* population, there is an advantage to collecting as much wild material as possible to achieve RN.

C10 Collect from wild plants the maximum seed over a number of years that is available, germinate and maintain.

C11 Collect from wild plants the maximum seed that is available, germinate and maintain. Consider (a) tissue culture of these 1st generation plants as a means to boost the ex situ population without relying on 2nd or 3rd generation plants and/or (b) supplement and replace as necessary with cuttings taken equally across all germinated plants within this stock. Only use *ex situ* sexual propagation if neither (a) nor (b) are viable options.

C12 Take cuttings, other plant parts or whole plants.

B. Which individuals to collect from?

There are two important issues here: (a) what to do if there is more than one population, and (b) specifically which individuals should be sampled?

Is collecting from more than one population a good idea?

For many critically endangered species this may be a moot point. There may be only one remaining population, or there may be so few individuals left that sampling the variation of all individuals is a priority and all attempts to reproduce the species are valid as emergency measures.

However, where more than one remaining population exists, difficult issues arise regarding when it is legitimate or worthwhile to collect and/or mix genotypes from these populations. These issues are usually case-specific, often complex, and occasionally controversial. A full consideration is beyond the scope of this guide, but practitioners might wish to consider the following key points.

> Assessing genetic diversity and difference can be undertaken in a laboratory using techniques such as isozymes or allozyme markers. It is not foolproof and requires material that is fresh or stored in liquid Nitrogen. If laboratory analysis is not available or affordable, careful field observation can be just as effective, if not more so.

1. If this is an issue that needs resolving for a species, it is valuable to research the *extent of genetic differentiation between potential source populations*. The less the difference the less the problem.

2. A focus should always be kept on collecting and maintaining genotypes *suitable for the eventual reintroduction site*.

3. For most species, most of the genetic diversity results from different genotypes *within* a population, with less provided by the differences *between* populations[xv]. This is even the case for species that exhibit strong geographic differentiation (unless they disperse pollen and seed locally and infrequently) and implies a reduced need to mix populations to boost variation. However this is a complex issue, refer to Falk et al (2001)[xvi].

4. Where additional and secure wild populations exist they can always be sampled at a later date to add their genetic diversity to a population being managed towards reintroduction, whereas it is almost impossible to remove genotypes once introduced and mixed.

5. Species on islands - where a disproportionate number of critically endangered species are to be found - are likely to be relatively prone to extinction through hybridisation[xvii], and thus particular caution should be exercised in such situations when introducing pollen or adult plants from related but distant populations.

6. Genetic considerations will in part depend on whether a species was *formerly common* or is *historically rare*. Oostermeijer[xviii] suggests that crosses between populations should be considered to increase genetic variation of small, isolated populations of *formerly common* species. For naturally *rare species* adapted to highly specific, geographically restricted habitats and for which every population often constitutes an important part of total genetic variation, he considers efforts should focus primarily on habitat protection and

management. Brigham (*ibid*) also suggests that populations that are historically rare may have adaptations to rarity that enable them to persist.

7. Priority should be given to populations most at risk.

The following table sets out some of the potential benefits and risks of mixing genotypes from more than one population.

Mixing genotypes from more than one population	
Value	**Risk**
Refreshment of genetically impoverished small populations, leading to greater resilience.	Hybridisation between populations can lead to extinction through: (a) influx of inappropriate genes, leading to an *ex situ* population less well adapted to local conditions than the original population, (b) leaking of sub-optimal genes into remaining endangered wild populations. (c) assimilation by a closely related taxon, perhaps blurring genetic boundaries between previously isolated taxa that are part way through a process of speciation. Note: Extinction of a pure native genotype can occur in *less than five generations* even when the 'invading' species and hybrids lack a competitive advantage[xix].
Hybrid vigour	'Hybrid' individuals may be vigorous individuals but with reduced fertility, and swamp endangered populations.
Can overcome inbreeding depression.	Loss of ecotypical distinctiveness, thus removing the option of placing reintroduced stock back into places to which they are specifically adapted.
	May destroy co-adapted gene complexes

Picking individuals within a population.

Ensuring diversity

It is important so far as possible to ensure adequate and appropriate genetic diversity in the *ex situ* population. The choice of material collected affects this genetic diversity, so a strategy is required to sample variation from the wild population. Where possible it is valuable to understand the patterns of genetic diversity in the target species. The viability of an *ex situ* population and its suitability for later reintroduction depends primarily on its *ecologically significant* genetic diversity. Therefore emphasis needs to be placed on collecting material from distinct individuals at different times and possibly different habitats.

It is possibly best to walk the site first before beginning collection to get a clearer impression of its limits and possible local environmental variation. Within the constraints of minimising or avoiding damage to the survival of remaining wild plants, generally speaking collect an equal amount of seed or cuttings from as many different, randomly-chosen[xx] individuals as possible (at least

> Make absolutely sure that the right species is being collected – similar species can be very deceptive!

50), including smaller and less vigorous plants with fewer seeds. Such low production may not necessarily reflect an overall lack of fitness, rather a lack of fitness in the current environment (a genotype that performs poorly one year may perform well in another). Bear in mind that the smaller plants may be less obvious. However, be wary of collecting plants that are infected with pathogens or viruses.

Where the population exhibits clear patterns of local environmental variation, sample randomly *within each variation*, collecting a proportion of the sample relative to the total population size. Bear in mind that factors such as different elevations and degrees of exposure can result in genetically different subpopulations.

If collecting plants or vegetative material, collect from as wide a geographic area as possible as it is often difficult to determine whether adult plants are genetically identical clones. On the other hand isolated plants should perhaps not be a focus of collection efforts if these plants are believed to be inferior as a consequence of self-fertilisation and/or inbreeding.

Keep collections from different plants *separate*, as for genetic reasons it may well be necessary in due course to ensure an equal contribution from each wild source plant to a future reintroduction attempt.

Collecting from more than one population?

If it has been decided to collect from more than one population, it may be best to collect from as many populations as remain. If a choice has to be made between source populations, it may be better to collect from the largest. There is evidence that these may retain more variation or produce the most vigorous material for an *ex situ* programme.

Existing collections?

Generally, existing *ex situ* collections of the species may prove a poor choice of material. Their provenance may be uncertain and if a short-lived species they may well have become genetically adapted to cultivation and thus they and their progeny less well adapted to future *in situ* conditions.

2.3 When and how often to collect?

Wherever possible sample over a number of years. This not only reduces impact on remaining wild populations, but potentially increases the diversity of seed collected (not every pollen parent may contribute to seed set every year). Spread-out collections can also be integrated with site and species monitoring programmes.

Also sample through an entire fruiting season, rather than all at once, to ensure capturing phenological variation and a mixture of self and outcrossed progeny.

2.4 Collecting techniques[xxi]

Maturity

Ensure seed is harvested when fully mature (i.e. achieved maximum natural dryness). Immature seed does not store so well. If seeds have to be harvested immature, initially dry them slowly, ideally over 2-5 days in their fruits.

Containers and bags

Seeds should be collected into permeable cloth or non-glossy paper bags, with plastic bags used to collect only fleshy fruits. If seed cannot be cleaned soon after collection, it will be necessary regularly to aerate the bagged fruit. If the plant has shed its seed and it has been collected from the ground, this should be recorded.

Cleaning and drying seed in the field

Seed should be cleaned as fully as possible in the field, and insects and fleshy material that may rot should be removed. If seed samples need to be dried, they can be placed inside a sealed container with blue silica gel (ratio of 2:3 seed to fresh silica gel, with minimal air) or dried rice, for example, as an effective substitute. Avoiding exposing seeds to light during drying, and it is certainly not advisable to dry in direct sunlight, as the heat may affect long-term seed viability. Beware microbial and fungal contamination at high humidities. A fungicide might be useful.

Temporary storage

Seeds should be kept dry and cool (but not below 10C) and should be transported to the seed bank as soon as possible. Simple methods such as keeping seed in the shade, and away from direct sunlight through windows of a vehicle may enhance long-term viability. If there are considerable diurnal humidity and temperature fluctuations, placing collecting bags into a large plastic bag at night will help to maintain an even temperature and relative humidity.

Collections have begun to be made. It's now crucially important to manage them carefully in order to maximise the prospects of a successful reintroduction, and minimise any damage to other species.

Step 3

How to maintain the species *ex situ*?

3.1 Choice of life stage to maintain.

3.2 Health and vigour.

Living plants.
Seed.
Tissue culture.
Dealing with low vigour material.
Security

3.3 Genetic diversity.

General issues
General advice
Domestication
Genetic drift
Inbreeding
Outbreeding
Inadvertent hybridisation

3.4 Increasing the *ex situ* population.

Seed, tissue culture or cuttings?

3.1 Choice of life stage to maintain

The choice of which life stage or stages will be maintained will depend on the species concerned and the resources available. The following table shows some of the major issues to consider when deciding what material to maintain *ex situ*.

	Living plants	**Seed**	**Tissue culture**
Advantages	Reintroduced living plants generally have better survival than reintroduced seeds. For slow-growing plants allows more rapid reintroduction of mature material should a potential reintroduction site become available.	Cheap for large quantities and wide genetic diversity. Potentially less risk of disease and other major threats. Valuable as an insurance policy *alongside* collections of long-lived species or slow-growing species that need to be increased by vegetative propagation.	Makes increase without domestication possible for species difficult to reproduce vegetatively. Potential for long term storage with minimal losses. Can restore vigour to weak material. Can provide material for reintroduction-focused research.
Disadvantages	Facilities, time and money required. Inadvertent selection pressures leading to domestication if *ex situ* sexual reproduction necessary. Risk of inadvertent introduction or escape of genes. Risk of disease spreading from plants or soil at reintroduction sites.	Some seed cannot be stored. A dead seed looks like a live seed - impossible to assess mortality without regular germination trials.	Cost. Specialist equipment and facilities required. Mutations can result.

3.2 How to maintain health and vigour in the collection

Living plants

Collecting and increasing knowledge

Cultivation techniques for some groups are fairly well known e.g. Orchidaceae and Cactaceae. ***Step 5*** will show where useful information may be found. For others there may be little or no knowledge of cultivation, and an observant, adaptive approach will be required that broadly mimics natural soil and other environmental factors, while alleviating to some extent the physiological stresses that may be found *in situ*. The extent to which wild conditions should be emulated is explored in more detail below.

Seed and cuttings from specimens *already in cultivation* may be useful in developing cultivation and germination techniques, but unless their provenance is very well documented such material should generally be carefully *excluded* from any breeding programme or reintroduction. Alternatively for very rare species, closely related species might be used for research, again taking great care to avoid hybridisation or escape.

Recording and sharing the results of such experimentation is particularly valuable.

When and to what extent should wild conditions be emulated?

In general the more different the *ex situ* environment relative to the wild environment, the greater the risk of domestication. So for species that need to be maintained for a number of generations it is important to mimic to some extent the wild habitat the species may be reintroduced to. This could extend to soil type and pH, exposure, day and night-time temperatures, etc., – indeed all aspects other than the major contributing factors to the species' *in situ* decline.

For species with a reasonable prospect of reintroduction within the reproductive lifespan of individuals in the *ex situ* collection, there may be advantages to providing more optimal conditions for growth. This could either help provide healthy vigorous stock for reintroduction and/or maximise the number, size and vigour of cuttings that may be part of the *ex situ* programme. Moreover, there are great benefits to boosting the *age at first reproduction* of plants to be used for reintroduction.

For some species particular attention will be required to necessary 'symbionts', for instance with terrestrial orchids experience has shown much greater success when using symbiotic propagation techniques.

Pests and Diseases

Obviously one way in which mimicking wild conditions may not be possible is the density of individuals in an *ex situ* collection. This may be much greater than *in situ* and this unnatural density may increase the risk of catastrophic spread of pests and diseases. Particular attention should be paid to monitoring this problem on a regular basis.

Also, be particularly careful to avoid and control pests and diseases, that might spread to wild populations either from the ex situ collection, or with reintroduced material[xxii].

Boosting vigour in weak material

Where stock is from old or senescent plants it may not be possible to achieve vigour simply by providing optimal environmental conditions. A number of techniques (other than reproduction of new plants by seed) may be tried to improve the situation.

Grafting.
In rare circumstance, grafting onto related species may be necessary to maintain some or the entire *ex situ* stock, for instance where rooting of cuttings is not sufficiently successful.

Growth promoters.
A number of proprietary mixes of hormones and vitamins claim to be able to revive damaged or dying plants.

Tissue Culture.
Tissue culture is perhaps the most reliable way of boosting vigour – see the section on Tissue Culture later in ***Step 3***.

Seed

Assessing viability

Just because a seed fails to germinate does not mean it is not viable. Many seeds can lie dormant for long periods until the right combination of factors stimulates them to germinate. Thus assessment of viability often cannot be undertaken until dormancy is fully understood.

Dormancy

Dormant seeds are those that do not germinate under any conditions when they are freshly matured. The following decision tree should help assess and understand dormancy.

D1 Do published literature or web searches provide useful information on dormancy and how to overcome it for members of the same family? Trial any recommended approaches. If successful go **D16**. If not return to this decision tree.
No useful information available? Go to **D2**.

D2 Weigh dry seeds, place on a moist substrate for 24 hours, blot dry, then reweigh. Does the weight show a significant increase? Go to **D5**.
No significant weight increase? Go to **D3**.

> If sufficiently accurate scales are not available, visible swelling of the seed may be sufficient indication of water absorption.

D3 Are the seeds large enough to cut a small hole? Make a small hole on the cotyledon end so as not to damage the root or shoot. Go to **D4**.

Seeds too small? Try rubbing gently between two sheets of sandpaper or 'acid scarification' if seeds are very plentiful. Go to **D4**.

In acid scarification, seeds are covered with concentrated sulphuric acid, gently stirred and allowed to soak from 10 minutes to several hours. When the seed coat has become thin, the seeds are removed, washed, and planted.

D4 Weigh dry seeds, place on a moist substrate for 24 hours, blot dry, then reweigh. Does the weight show significant increase? Go to **D5**.
No significant weight increase? Go to **D6**.

D5 Do the seeds now germinate? Go to **D16**.
Still no germination? Go to **D6**.

D6 Does the species normally germinate in the wild after a cold period? Go to **D7**.
Does the species normally germinate in the wild after a hot period? Go to **D8**.
Species does not normally germinate in the wild after either a cold or hot period. Go to **D9**.

Different species require different lengths of time and temperatures so a degree of experimentation may be required.

D7 Place the seeds on a moist substrate at about 5°C for a period of 8-16 weeks. Go to **D9**.

D8 If germination normally takes place following a hot period, place the seeds on a moist substrate at about 20-25°C. Go to **D9**.

D9 Do the seeds now germinate? Go to **D16**.
Still no germination. Go to **D10**.

D10 Is the species from an ecosystem where fire may play a more or less regular part in ecosystem processes? Go to **D11**.
Species not from a fire ecosystem? Go to **D12**.

D11 Try high temperature or smoke-related techniques. Immerse seeds in boiling water for a few minutes or expose to smoke[xxiii]. Go to **D14**.

D12 Is the species parasitic or saprophytic or a member of the Balanopharaceae, Burmanniaceae, Ericaceae, Gentianaceae, Hydnoraceae, Lennoaceae, Monotropaceae, Orchidaceae, Pyrolaceae or Rafflesiaceae? Go to **D13**.
Species not as above? Go to **D15**.

D13 Seeds may have undifferentiated embryos requiring exudates from the roots of potential host plants. Try germinating in the presence of such hosts' roots or consult expert advice on the genus or family. Go to **D14**.

D14 Do the seeds now germinate? Go to **D16**.
Still no germination? Go to **D15**.

D15 Keep trying, be patient. Reconsider alternative means for maintaining the species *ex situ*.

D16 Germinate a sample of seed spread evenly across the seed accessions. Record techniques used, germination and survival rates.

Viability and longevity

To maintain maximum viability, ideally seed should be tested every three years, and grown on to provide fresh seed stock when viability drops below 85% of that for fresh seed. However when regenerating a population this way there is a significant trade-off between maintaining viability and the implications of domestication. Moreover, assessing viability with any degree of accuracy requires a remarkably large sample size. And if you're keeping all maternal seed stocks separate, the implications are considerable. There are no specific solutions to this problem and it will need to be assessed on a case-by-case basis. However, the larger the initial collection, the more will be available for viability testing.

A useful guide to testing longevity has been produced by the Millennium Seed Bank Project[xxiv] and the Association of Official Seed Analysts produces useful Rules for Testing Seeds[xxv].

Note. For seed collections, until all germination cues are understood, the true viability or genetic diversity of a seed collection cannot fully be assessed.

Storage

In the absence of any other indications aim for 15-25% Relative Humidity (12% seed water content), and –18^{o}C (the temperature of a standard freezer). However optimum seed storage conditions vary considerably from species to species. Fortunately there are useful sources of information. An online database of storage information for over 7000 species from 251 families is held at the International Plant Genetic Resources Institute[xxvi]. A useful summary of relevant issues and information can also be found in Guidelines for Seed Storage (2004) by Christina Walters[xxvii].

Keep seed from each maternal parent separate to make it possible to equalise the contribution of different parent plants in reintroduction.

Avoiding exposing seeds to light during storage. Also beware microbial and fungal contamination at high humidities. A fungicide might be useful.

Tissue culture

In vitro technology (tissue culture) is the rapid and large-scale laboratory propagation of genetically identical plant material in glass or plastic jars. The objective is to accelerate multiplication as well as obtaining disease-free material. It has developed to such an extent that tissue culture is now considered relatively 'low-tech' and routine[xxviii].

Security

Where the *ex situ* facilities may be at risk from spread of disease, natural catastrophe or political upheaval, serious consideration should be given to splitting the *ex situ* collection between two or more locations, or better still maintaining duplicate material in other facilities.

3.3 How to maintain or develop sufficient and appropriate genetic diversity

General Issues.

Major genetic problems can affect the *ex situ* population. In summary these are:

* A narrowing of the genetic diversity available for reintroduction. Not only does this reduce the robustness of the reintroduced population but may increase the adverse effects of inbreeding after reintroduction.

* A shift of genetic diversity towards what is well adapted to cultivation but poorly adapted to wild conditions.

* A spread of genetic diversity (perhaps from mixing the gene pools of different wild populations) resulting in many individuals less well adapted to the requirements of a specific reintroduction site.

Maximum genetic diversity is thus not always the right goal; rather it should be maximum genetic diversity *appropriate to a specific reintroduction site or sites*. In other words the *ex situ* collection should be striving to maintain and/or assemble a genetically appropriate, reintroducable population that can function in a specific *in situ* environment.

This is a complex task, and this section attempts merely to highlight and summarise the important issues and provide some guidance on how to avoid the worst effects.

General Advice

Although specific genetic issues need to be considered and managed in different ways, four key approaches cover most of the problems likely to arise:

1. Avoid sexual propagation in favour of vegetative propagation.

2. Maintain as large an effective *ex situ* population as possible.

3. Prevent hybridisation with closely related species that are either also being kept *ex situ* or occur in the local environment.

4. Equalise reproductive contributions from all wild-collected sources.
   This requires that when plants or plant parts are taken from the wild, populations be maintained separately in the *ex situ* collection, in order to keep track of offspring production. Likewise where wild-set seed is germinated *ex situ*, keep seedlings from each maternal parent separate.

Given the many genetic problems that can arise with *ex situ* collections, it may be worth considering keeping the collection *within* the natural habitat (‘*inter situ*’). Such conditions would to some extent eliminate mutations and genotypes that would prove poorly adapted in a subsequent reintroduction. However care must be taken to avoid the threats that have led to the species becoming critically endangered in the first place.

Domestication.

For species that have to be regenerated more than once from seed, domestication can be an acute problem.

Inadvertent or unavoidable selection pressures can remarkably quickly produce an *ex situ* population that is very different from its wild ancestors and very poorly adapted to wild conditions. This is especially true for species that need to be maintained in cultivation by sexual propagation. Moreover there is evidence that species often respond very swiftly to selection pressures in cultivation, in just the same way that they might respond to a new or significantly changed environment in the wild.

Unless germination and survival reach 100%, second and third generation plants from cultivated source material will likely comprise genotypes that rapidly diverge from natural populations, resulting from the non-natural selection pressures of non-natural growing conditions.

Hence the emphasis for short-lived plants should be seed storage rather than cultivation. Note however that genetic change leading to domestication can even occur with seed *storage*. Seeds differ in the strength of their dormancy and tolerance to storage conditions.

How to avoid

* Where possible avoid *ex situ* sexual propagation.

* Where sexual propagation is essential to increase numbers, the best response would probably be to maximise the effective population size during all phases although constraints on resources and facilities may make this impracticable.

* Maintain maternal lines separately to allow equalising of 'family size'.

* Maximise generation time (thus minimising the number of generations between collection and reintroduction) or better still only use one generation – germinated and grown on specifically for reintroduction.

* Attempts to minimise domestication should take care not to simultaneously reduce the adaptability of the reintroduced population through keeping the genetic pool 'pure but narrow'. There may be some advantage to allowing some increase of genetic variation because we do not know which genotypes will be the most successful and we want the reintroduced population to have as much evolutionary potential as possible.

Genetic drift

Genetic drift is the random loss of genetic information from small populations, and the 'fixing' of particular characters as a result. This gives a gene pool that is narrower and hence less flexible and less able to adapt to subsequent *ex situ* conditions.

How to avoid

* Using seed storage or vegetative propagation where possible.
* Maximise generation times.
* Keep population sizes as large as possible.
* If possible refresh genetic diversity regularly with new genetic material. Only a small number of new individuals, maybe 1-5 per generation may be effective.
* Equalise the genetic contribution from each individual to subsequent generations, including equalising the *pollen* contribution from each individual. This may require temporarily isolating 'dominant' males (i.e. those producing most pollen), and may require hand pollination when natural pollinators are absent.
* Equalise sex ratios in dioecious species.
* Ensure progeny from both fast and slow-germinating seed are reflected in the *ex situ* population.

Accumulation of mutations

In *ex situ* populations the threats to the wild population are (hopefully!) reduced or removed, and the competition for resources (water, nutrients) are also likely reduced. Consequently, deleterious mutations - that would probably be removed in the wild – can remain and accumulate in the *ex situ* population.

Maintaining an *ex situ* collection to keep potentially valuable variation while eliminating definitely deleterious mutation is an extremely difficult balance to achieve[xxix]. Some 'deleterious' mutations may be beneficial under different circumstances. However, in most case where sexual propagation is required, the risks of domestication probably outweigh those of accumulation of mutations.

How to avoid

* Increase generation time.
* Consider mimicking natural selection regimes to some extent, in other words a mild selective regime designed to reduce the accumulation of deleterious mutations.

Inbreeding

Inbreeding 'depression' can come about through self-fertilisation and crossing between close relatives, resulting in the 'fixing' of some characteristics in the *ex situ* population. This may be a particular problem when sexual propagation is required to maintain a very small *ex situ* population. In such situations it is difficult to maintain genetic diversity even with a careful plan.

How to avoid

* Maintain as large an effective population as possible

* So far as possible, maintain maternal lines separately to allow equalising of 'family size'.

Outbreeding

While maintaining maternal lines separately may be recommended for many of the issues summarised above, there may be times when mixing the genotypes of plants from different locations is deliberately undertaken. This may be in an attempt to increase genetic diversity, or to avoid inbreeding depression or simply as the only practicable way to increase the *ex situ* population where only very small numbers of self-infertile plants are available. However, this approach should be taken with some caution as outbreeding may inadvertently reduce the fitness of offspring in the particular locations to which they may be subsequently introduced.

Outbreeding 'depression' is the loss of adaptive traits through crossing plants with considerable adaptive genetic difference (or with different co-adapted gene complexes). Such difference may occur with individuals adapted to microclimatic conditions as little as 100m apart. The impact of such outbreeding may not be noticeable for a number of generations.

How to avoid

Keep maternal lines separate wherever possible, but if different maternal lines have to be crossed:

* Take particular caution where there are obvious differences between populations.

* Keep 'habitat lines' separate if possible to allow subsequent reintroduction to suitable similar habitats – ecological matching of parents is more important that geographical matching.

In reality it may be difficult to avoid the negative impacts of outbreeding, but these will hopefully be outweighed by the benefits.

Inadvertent hybridisation

In extreme cases deliberate hybridisation can be used as a conservation tool to restore fertility for a species that is no longer producing seed, or to confer resistance to a species threatened by an introduced pest or disease. However, more often hybridisation will be accidental and can pose serious problems not only to the critically endangered species but also to ecosystems in which the hybrids become established.

Ex situ collections may bring into close proximity species that do not normally occur together (either within the collection or in nearby natural habitat) bringing with them the risk of hybridisation.

The strength of barriers to hybridisation varies from species to species, but it may be that some endangered species are particularly susceptible to hybridisation. For instance barriers to hybridisation are known to be limited in many oceanic island plants.

Spontaneous hybridisation can result in:

* Contamination of *ex situ* populations. For species with weak reproductive barriers this can lead to extinction within as few as five generations[xxx].
* 'Leakage' of genes into wild populations of the same or related species, either directly or via naturalised escapes.
* Generation of novel invasive hybrid taxa, either *ex situ* or after escape.

Hybridisation has been shown to be an important stimulus to the evolution of invasiveness - and *ex situ* collections have often been implicated as sources of invasive plants. The manager of an *ex situ* population needs to look beyond the bounds of their collection to consider possible invasive hybrids arising form all combinations of cultivated *ex situ* stock, naturalised escapes, and wild populations. **Appendix D** presents management guidelines to reduce these risks, proposed by Maunder et al (2004). These are especially important for collections of open-pollinated species with seedlings destined for reintroduction.

How to avoid.

* Isolate reproductively active members of the *ex situ* population from closely related species both within the collection and in nearby habitats.

* Monitor the collection and nearby habitats for appearance of hybrids.

3.4 Increasing the ex situ *population*

There are many factors that influence a choice of how to increase the *in situ* population. The following set of choices is based on three assumptions:

1. **Seed collection is preferable to collection of plants (less impact, greater volume).**
2. **Seed storage is preferable to maintaining and reproducing plants ex situ (lesser cost of maintenance, avoids domestication, etc.).**
3. **Reintroduction of living plants is preferable to seed (better establishment rates).**

1st choice: Wild-set seed, stored then germinated and grown on to provide RN plants to coincide with *in situ* conditions becoming suitable for reintroduction.

RN = Number required for reintroduction

2nd choice: Wild-set seed, germinated and maintained as RN living plants until *in situ* conditions become suitable for reintroduction.

3rd choice: Wild set seed, germinated and maintained as living plants, supplemented up to RN by cuttings[xxxi] from wild plants.

4th choice: Wild set seed, germinated and maintained as living plants, supplemented up to RN by cuttings from as many resulting *ex situ* plants as possible.

5th choice: Cuttings from RN of different wild plants maintained as RN living plants until *in situ* conditions become suitable for reintroduction.

Wild-set seed can and often should be collected over a number of years (refer to ***Step 2***).

6th choice: Cuttings from as many different wild plants as possible supplemented up to RN by cuttings from as many resulting *ex situ* plants as possible.

Notes:

Species with wild-set seed available that is neither storable nor produces long-lived plants present a particular challenge. For these the best approach may be to maintain as far as possible by cuttings from *ex situ* plants, supplemented only where essential by plants from *in situ* seed collection, and using techniques to minimise domestication and preserve appropriate diversity.

If insufficient storable, wild-set seed is available, *in vitro* methods can be very useful for increasing stock. Likewise for species that are very difficult to propagate or reduced to a single or very few individuals, especially if seed set is minimal.

Specific pollinators may be absent from *ex situ* collections, thus if sexual propagation is chosen for maintaining or increasing the population, hand pollination may be required. Indeed this may be desirable anyway to manage genetic diversity.

Step 4

Moving towards reintroduction.

4.1 Reintroduction guidelines.

4.2 Reintroduction-focused research

4.3 Where?

4.4 When?

4.5 Care after reintroduction?

4.6 Education and Awareness

4.1 Reintroduction guidelines.

The IUCN Species Survival Commission Re-introduction Specialist Group has produced a set of Reintroduction Guidelines (IUCN, 1998) to which the reader is directed. These guidelines cover diverse issues but of most relevance here is *Guideline 4a (v), Availability of Suitable Release Stock*, reproduced in **Appendix B**.

4.2 Reintroduction-focused research

Collection, maintenance, increase and reintroduction will inevitably be somewhat experimental and adaptive in nature, and thus all are opportunities to gain experience.

Given the obvious conservation value of the *in situ* and *ex situ* populations a cautious approach will generally be wise, guided wherever possible by careful research. Such research could for instance determine the optimum life stage for reintroduction. Success rates for reintroduction tend to be higher for plants than seeds, though mature plants may not transplant so successfully as seedlings. Trials are advisable.

If choosing between possible new sites with genetically identical stock available, planting out trials could provide very useful information to determine the sites most likely to be appropriate.

Ex situ material can also make important contributions to understanding the reproductive biology and ecology of the species. Such research must be carefully planned to avoid any adverse impacts on the potential vigour and size of *ex situ* populations intended for reintroduction.

Reintroductions can entail long-term commitments, particularly for longer-lived species that may take many years to reach reproductive maturity. Monitoring (and probably management) of reintroduced populations will be on-going responsibilities.

For species extinct in the wild and where no viable propagation is being achieved *ex situ*, an experimental reintroduction approach may give the opportunity to study the species more closely, or may allow some ecological conditions or processes that are missing from *ex situ* conditions.

4.3 Where?

The question of where to reintroduce a species is complex but the considerations summarised below fall into two broad areas – which general area (the 'macro' issues) and exactly where within the right habitat (the 'micro' issues).

The 'Macro' Issues

The old site or a new one?

In some ways reintroduction to the original site has major benefits:

(a) The site is at least known to have had the potential in the past to support the species.
(b) The site may allow the species to resume its ecosystem function and evolutionary path. For instance augmentation of remnant individuals could be critical for conservation of invertebrate species that depend on the plant species being reintroduced.

However, it may be that the species' original locations can no longer be expected to provide long-term suitable conditions. Climate change will almost inevitably affect a possible reintroduction site, and natural barriers or habitat fragmentation may well cut off opportunities for the species (and the communities on which it might depend) to adjust or move with time.

For an original site the following issues may need consideration:

* The original site was clearly imperfect for the species otherwise it would not have become critically endangered. Have the threats been adequately and dependably reduced?

* The direct and indirect implications of climate change on habitat and important species therein must be seriously considered. Climate change may render the site even less appropriate for the species than before. Maybe by the time reintroduction is a possibility the ideal site will be at some distance from the original collection site.

* If the old site has remnant individuals genetic implications need considering, as well as pathogen implications.

For a new site the following issues may need consideration:

* Any reasons for an absence of natural colonisation in the past.

* Novel interactions and responses are likely. For instance, is there a risk of introducing disease or pathogens with plants or soil, or the possibility of hybridisation with a closely related species? This could either be damaging to the survival of the introduced species, or related species, or result in an invasive hybrid. Refer to ***Step 3*** for more on this important issue.

* Is inoculation with the correct root-nodule firming bacteria required?

How many sites to reintroduce to?

Where the number of individuals available for reintroduction is limited, there is a trade-off to consider between more reintroduction sites perhaps reducing risks to overall survival and genetic diversity, but increasing these risks to each individual population. Trials and research may be required.

Security

The security of a site can be related to security of tenure, or predictability of management or other external factors. All need considering

Tenure

* Persistence of the reintroduced population may depend on stability of tenure and management of the reintroduction site. Management and monitoring are likely to be long-term responsibilities. Legal ownership and both degree and security of protection are thus critical issues. Some authorities have advised at least 50 years security of tenure. Consider whether a covenant or conservation agreement with the landowners could be valuable.

Management and External Threats

* Is the site large enough to support a self-sustaining population over the long-term?

* Are there any threats to the species at the site, and can they be managed or removed? Bear in mind treats that may not be immediately apparent such as soil borne fungal diseases or changes to the water table.

* Is land management at the site – *or neighbouring areas* - likely to remain compatible with the species?

The 'Micro' Issues

Ecological considerations

* Take great care to choose the correct local microclimates for the life stage reintroduced, ideally local microclimates that match those of the original collection for that material. Local adaptations may sometimes be important, even on a very fine scale. Also ensure the species is reintroduced to habitat at the correct successional stage.

* Find locations that match exactly the local landscape and habitat features of individuals of the original population/s and so far as possible locations *that match the individuals from which the specific reintroduced specimens originate*. This will include successional stage, aspect, slope, degree of shelter, shade, soil water conditions and variability, mycorrhizal associates, soil texture, pH and chemistry. Many introduction failures have been related to inappropriate of soil characteristics.

* Bear in mind the habitat/s required by the species' pollinators and dispersers. Also consider potential competitors and herbivores.

* Bear in mind broad ecological and environmental factors, e.g for wind-dispersed species plant out where prevailing winds give good prospects for dispersal.

* Also bear in mind more specific interactions with mutual or symbiotic species or host species.

* Consider locating near species that share a pollinator.

Distribution and Layout

* Be aware of potential herbivory and disease impacts that could result from unnaturally dense aggregations of individuals following reintroduction. Single plants or small groups have been shown (eg Kareiva 1985[xxxii]) to be less affected.

* On the other hand clumps may be necessary to attract pollinators or seed dispersers.

* A random layout amongst natural vegetation could make plants hard to find and hamper monitoring, and it may be easier to plant more or less in rows. Or following contours, using appropriate gaps in vegetation. Use of rows could also make monitoring of any experimental approaches more straightforward.

* If clonal material is being used, lay out plants to maximise chances of cross-pollination between clones.

* If planting is to take place over a number of years, leave gaps to fill art a later date.

4.4 When?

* Successful reintroduction depends on a healthy, intact, functioning ecosystem to return to.

* Reintroduction may also depend on a sufficiently large reintroduced population (RN). Losses of as much as 99% may be suffered before a reintroduced population begins to establish and increase in size. Also to achieve the fastest adaptive response it is best to plant out as many as possible at once rather than reintroducing *ex situ* material over time. However it may be vital to keep back *ex situ* stock as insurance!

* Beware reintroduction during environmental or climatic extremes (drought, etc.) that might drive the population down below a viable level before it has a chance to establish and reproduce.

* If the species occurs in populations of mixed ages or a number of discrete ages, consider staggering the reintroduction over a number of years to mimic natural

populations. This may also reduce the risk of failure due to unfavourable conditions for establishment.

4.5 Care during and after reintroduction?

All plants need to be uniquely and securely labelled. Ensure labels can withstand environmental extremes, fire and any other risks that may affect the site.

Extreme care should be taken to minimise stresses during any transplanting, and any damage to the site. Consider 'hardening off' plants to be transplanted, maybe by placing the plants in a semi-protected location within the habitat to which they will subsequently be transplanted, to allow slow adjustment to *in situ* conditions.

To aid establishment try to use plants that are not pot-bound, consider pruning leggy growth and removing any flowers and fruit to reduce damage during transport and excessive transpiration after planting.

Almost certainly some degree of short-term intervention to aid establishment will be necessary. Herbivores may constitute a threat to many freshly introduced plants, so some degree of protection may be worth considering. Likewise, provision of shelter, water, nutrients and possibly mulching to provide optimal relative humidity and temperature might be considered. Hand pollination or seed dispersal may also be required until natural agents have also recovered and are able to play the role. However, ensure that intervention does not adversely affect other species.

It should of course be borne in mind that care after reintroduction will to some extent slow down the necessary adaptive response of the new population to its environment.

Long-term care and management of the reintroduction site may well be necessary but goes beyond the scope of this guide.

4.6 Education and Awareness

Developing support and understanding for the conservation and reintroduction initiative may prove to be vital for its short and long-term success. The following suggestions are adapted from *A Handbook for Botanic Gardens on the Reintroduction of Plants to the Wild*[xxxiii].

* Explain the economic, cultural, historic and biodiversity values of the critically endangered species to government, sponsors, local communities and the media.

* Involve local communities, assess attitudes and raise awareness within local communities of the reasons why the species became critically endangered in the first place, hopefully helping to reduce future threats.

* Attract and motivate volunteers to act as monitors, wardens or guides, both at the *ex situ* collection and possibly at reintroduction sites. This may be especially valuable if plant collecting was or remains a threat.

* Develop educational materials for local use, but also share them more widely through networks to aid and guide other initiatives. Consider schools projects.

Step 5

What to Record, Report and Monitor?

5.1 Why Record and Monitor?

5.2 Why Report?

5.3 When?

5.4 What to Record and Monitor?

5.5 Where to Keep the Records?

5.6 Where to Report the Data?

"No careful attempts at reintroduction are too shallow, no innovations too simple, and no lessons too apparent to go unsummarized and unreported. There is no other way to get this young science off the steep slope of the learning curve".

Bruce M. Pavlik

5.1 Why Record and Monitor?

Recording, monitoring and reporting are not additions to the process, they are an integral part of it. The amount of data that needs to be recorded and maintained may seem burdensome, but many reintroductions and transplantations have been failures, not just because plants have died or failed to reproduce, but because projects failed to keep detailed notes. And as a result much of the knowledge that was or could have been gained has been lost.

5.2 Why Report?

Given the intensity of threats and the number of species thy affect, there simply is not time to continually 'reinvent the wheel', even if there were sufficient resources. And experience gained from failures is just as important as for successes.

5.3 When?

Monitoring of wild populations is required before, during and for a very long time after collections for *ex situ* management and reintroduction. Following the fate of individual plants can provide useful data and insights.

5.4 What to Record and Monitor?

In situ *biological and ecological data*

Information about the biology and ecology of the species in the wild will be invaluable in guiding reintroduction plans. Such information might include:

- Collector(s) name, number and institute.
- Date.
- Latitude, longitude, map references, etc.
- Full habitat details – at both the macro and micro scales.
- Maturity, physical characteristics and numbers of populations and individuals.
- Any threats noted, presence of herbivores, pathogens, parasites, etc.
- General health and vigour of individuals, including seed-set, insect damage or grazing by herbivores. Malformed flowers or other organs and few or empty seeds may be evidence of inbreeding depression in smaller populations.
- Microclimatic data.

Microclimatic data

Microclimatic data should ideally be recorded *for each wild individual from which samples are collected*. This will allow careful selection of specific sites for reintroduction of their progeny. This data could cover parameters such as:

* habitat successional stage (e.g. average height and density of vegetation,
* aspect,
* slope,
* degree of shelter,
* shade,
* soil water conditions (including drainage) and variability,
* mycorrhizal associates,
* soil pH and chemistry.

Records of the collection

A very worthwhile summary of how best to keep plant records for living collections can be found in The Darwin Technical Manual for Botanic Gardens[xxxiv]. Botanic Gardens Conservation International also produces a computer database, BG Recorder, suitable for maintaining records of an *ex situ* collection. This database is available to its members. See ***Step 6***.

As is shown in ***Step 3*** maintaining separate 'lines' within an *ex situ* collection can be very important. So use particular care with plant labels – attach one to the pot or soil and another to the plant where possible. Plant health records are very important.

Horticultural techniques and outcomes

This data will be of great value not only for developing and refining storage, maintenance and population increase techniques for the target species but increasingly for closely related species that may require similar initiatives in the future.

After reintroduction

Every reintroduction plan should determine the criteria by which success should be judged – generally in terms of abundance, extent, persistence over time, and resilience. These should be used to guide the selection of parameters for recording and monitoring. These parameters are likely to include:

* survivorship,
* disease,
* pathogens,
* health,
* seed viability
* genetic status,
* growth,
* age to maturity, and
* reproductive success and nature, and
* the effects of post-reintroduction management.

For a new site the appearance of any hybrids should be closely monitored. Swift action may be required, including removal of the hybrids and introduced plants.

Every reintroduction is also an opportunity to study the 'founder effect', and its impacts on genetics, population dynamics and reproductive biology.

Natural populations

If natural populations of the species remain, parallel monitoring of these should be undertaken to provide very useful comparisons with reintroduced populations.

5.5 Where to Keep the Records?

While all initiatives will naturally want a copy of all their data to hand, don't forget to consider data security – maintaining a regularly updated copy of all data at a different location should be a routine action.

What makes good recording and monitoring?

There is more to monitoring than might at first be imagined. The following issues are some of those needing to be considered. For more on this topic refer to Sutter R D (1996) [xxxv] and Vallee L et al (2004)[xxxvi].

* Monitoring data must have a known, and acceptable level of precision.
* Data collection techniques need to be repeatable over many years and by different people.
* Data must be collected over a long enough period of time to capture the effects of important natural processes and responses to management.
* Monitoring techniques must be sufficiently cheap and simple to be maintained. Decision on which parameters to monitor may require a large set initially, refined to a smaller set of valuable but easily measured parameters.

5.6 Where to Report the Data?

As yet there is no single co-ordinated focus for reporting on 'intensive care' and reintroduction initiatives for endangered plant species. It is hoped that these guidelines and the website on which they appear www.sustainablehorticulture.org will stimulate sufficient interest and debate that an appropriate forum will emerge to fill this vital role.

Until this is the case, reporting on initiatives should certainly take place locally but perhaps also be sent to the Alliance for Sustainable Horticulture, the IUCN Reintroduction Specialist Group, and Botanic Gardens Conservation International (see ***Step 6***).

Step 6

Where to Turn for Help and More Information?

6.1 Information on species

6.2 Propagation and cultivation manuals.

6.3 Other published guidelines.

6.4 Publications

6.5 Institutions and Individuals

6.1 Information on species

The Botanic Gardens Conservation Website contains valuable and relevant information on many thousands of species, including where they may be held in cultivation and thus where information on propagation may be available.

6.2 Propagation and cultivation manuals

There are many such manuals in publication. One produced specifically for botanic gardens that may have relatively few resources is The Darwin Technical Manual for Botanic Gardens by Etelka Leadley & Jane Greene (eds, 1998) BGCI. English French and Spanish versions are available.

6.3 Other published guidelines

- US Fish and Wildlife Service *Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act*. US Federal Register. September 20, 2000. Volume 65, No.183.

- The Nature Conservancy's *Practical Handbook for Population Viability Analysis*[1].. Morris M *et al* (1999).

- The IUCN Species Survival Commission Re-introduction Specialist Group has produced a set of Reintroduction Guidelines (IUCN, 1998) to which the reader is directed. These guidelines cover diverse issues but of most relevance here is *Guideline 4a (v), Availability of Suitable Release Stock*, reproduced in Appendix B.

- IUCN SSC also produces *Guidelines for the Management of* Ex Situ *Populations*.

- *Guidelines for the Translocation of Threatened Plants in Australia.* Vallee T, et al (2004). Australian Network for Plant Conservation, Canberra.

- Flora Bank Guidelines 1: Native Seed Storage for Revegetation. www.florabank.org.au

6.4 Publications

Edward O. Guerrant, Kayri Havens, and Mike Maunder (2004) ***Ex Situ Plant Conservation: Supporting Species Survival in the Wild.*** Society for Ecological Restoration International with Island Press.

[1] Morris M *et al* (1999). A Practical Handbook for Population Viability Analysis. The Nature Conservancy.

Falk D A et al (Eds) (1996) ***Restoring Diversity – Strategies for Reintroduction of Endangered Plants.*** Center for Plant Conservation with Island Press.

Brigham C A & Schwartz W S (Eds) (2003) ***Population Viability in Plants: Conservation, Management and Modeling of Rare Plants.*** Springer-Verlag, Ecological Studies Series.

Walters (2004) ***Guidelines for Seed Storage.*** In Edward O. Guerrant, Kayri Havens, and Mike Maunder (2004) Ex Situ Plant Conservation: Supporting Species Survival in the Wild*.* (Society for Ecological Restoration International with Island Press).

Association of Official Seed Analysts (1999) ***Rules for Testing Seeds*****.** Lincoln, Nebraska. Online information at: http://www.aosaseed.com/reference.html

Frankham R *et al* (2002) ***Introduction to Conservation Genetics.*** CUP.

P Sjogren Gulve & T Ebenhard (Eds). 2000. ***The Use of Population Viability Analyses in Conservation Planning.*** Blackwell Science, Ecological Bulletin 48.

Journal of ***Conservation Genetics.*** Kluwer Academic Publishers.

Brigham CA & Schwartz MW (Eds) (2003). ***Population Viability in Plants – Conservation, Management, and Modeling of Rare Plants.***

Morris W & Doak D (1999) ***A practical handbook for population viability analysis.*** The Nature Conservancy Press, New York.

Guerrant EO & Pavlik BM (1997) ***Reintroduction of Rare Plants: Genetics, Demography, and the Role of* Ex Situ *Conservation Methods***. In Fielder PL & Kareiva PM, Conservation Biology for the Coming Decade. 2nd Edition. Chapman & Hall.

Smith R D *et al* (2003) ***Seed Conservation – Turning Science Into Practice.*** Royal Botanic Gardens, Kew.

Vallee L et al (2004) ***Guidelines for the Translocation of Threatened Plants in Australia.*** Australian Network for Plant Conservation, Canberra.

6.5 Institutions and Individuals

Alliance for Sustainable Horticulture

ASH sets out to bring interested parties together to help horticulture make a bigger contribution to sustainability and to help make horticulture sustainable itself – environmentally, socially and economically. ASH is hosting this guide and discussions about how it should develop.
www.sustainablehorticulture.org
Tel: +44 (0) 7905-795170

Botanic Gardens Conservation International

BGCI builds and maintains a world network of Botanic Gardens for plant conservation, and works to educate and promote conservation awareness and sustainability by providing technical guidance, data and support for Botanic Gardens in over 150 countries worldwide.
www.bgci.org.uk
Descanso House, 199 Kew Road, Richmond, Surrey, TW9 3BW, UK
Tel: +44 (0)20 8332 5953
Fax: +44 (0)20 8332 5956

The Eden Foundation

The **Eden** Foundation underlies the public face of the Eden Project, directing the broader scientific, research and education programmes that further the Eden Project mission and helping to define the philosophy and direction of Eden. In short, building on the achievements of Eden as a visitor experience, the Eden foundation is developing as a centre of excellence for research and teaching in science communication.
www.edenproject.com
Eden Project, Bodelva, St Austell, Cornwall, PL24 2SG, UK
Tel: +44 (0)1726 811911
Fax: +44 (0)1726 811912

International Plant Genetics Institute

IPGRI is an international research institute with a mandate to advance the conservation and use of genetic diversity for the well-being of present and future generations.
www.ipgri.cgiar.org
Via dei Tre Denari 472/a, 00057 Maccarese (Fiumicino), Rome, Italy.
Tel: +39 (0)6 6118.1
Fax: +39 (0)6 61979661

International Union for the Conservation of Nature

IUCN exists to "*influence, encourage and assist societies throughout the world to conserve the integrity and diversity of nature and to ensure that any use of natural resources is equitable and ecologically sustainable*."
www.iucn.org
Rue Mauverney 28, Gland 1196, Switzerland.
Tel: +41 (22) 999-0000
Fax: +41 (22) 999-0002

IUCN Reintroduction Specialist Group

The **RSG** is one of the IUCN Species Survival Commission's specialist groups, and exists to develop and promote inter-disciplinary scientific information, policy, and practice to establish viable wild populations in their natural habitats.
www.iucnsscrsg.org

Frédéric Launay, Assistant Secretary General Science & Research, ERWDA, P.O. Box 45553, Abu Dhabi, UAE
Tel: +971-2-681-7171
Fax: +971-2-681-0008

WWF

WWF is a global organisation dedicated to conservation of the natural world, acting locally through a network of family offices. All these offices do all they can to halt the accelerating destruction of our natural world.
www.panda.org

Millennium Seed Bank Project

The **MSBP** is an international collaborative plant conservation initiative. This worldwide effort aims to safeguard 24,000 plant species from around the globe against extinction, including 10% of the world's dryland seed-bearing flora. The MSBP also seeks to improve seed conservation technology, and make seed available for research and reintroduction.
www.rbgkew.org.uk/msbp/
Millennium Seed Bank Project, Wakehurst Place, Ardingly, Haywards Heath, West Sussex, RH17 6TN, UK.
Tel: +44 (0)1444 894100
Fax: +44 (0)1444 894110

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[i] IUCN Red List of Threatened Species™ , 2004. http://www.redlist.org/
[ii] Maunder M *et al* (2004). Ex Situ methods: A Vital but Underused Set of Conservation Resources. In Guerrant E O *et al* (Eds) Ex Situ Plant Conservation – Support Species Survival in The Wild. Island Press.
[iii] Pavlik, Bruce M (1996) Defining and Measuring Success. In Restoring Diversity – Strategies for Reintroduction of Endangered Plants, Falk Donald A *et al* (Eds) Island Press, Washington DC.
[iv] In this context a 'controlled environment' is one manipulated for the purpose of producing or rearing progeny of the species in question, and of a design intended to prevent unplanned escape or entry of plants, or gametes, embryos, seeds, propagules, or other potential reproductive products.
[v] Observed, inferred or projected.
[vi] Observed, estimated, inferred, projected (or suspected for A22), based on any of the following: (a) direct observation, (b) an index of abundance appropriate to the species, (c) a decline in area of occupancy, extent of occurrence and/or quality of habitat, (d) actual or potential levels of exploitation, (e) the effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.
[vii] Menges E S et al (2004) Effects of Seed Collection on the Extinction Risk of Perennial Plants. In Ex Situ Plant Conservation. In Guerrant E O *et al* (Eds) Ex Situ Plant Conservation – Supporting Species Survival in The Wild. Island Press.
[viii] FloraBank (1999). Guidelines 6: Native Seed Collection Methods. FloraBank, Canberra.
[ix] NB Some species may have adapted to a high level of inbreeding and thus not require higher minimum populations for viability.
[x] Guerrant E O and Fielder P L (2004). Accounting for Sample Decline during Ex Situ Storage and Reintroduction. In Guerrant E O *et al* (Eds) Ex Situ Plant Conservation – Supporting Species Survival in The Wild. Island Press.
[xi] Towill L (2004). Pollen Storage as a Conservation Tool. In Guerrant E O *et al* (Eds) Ex Situ Plant Conservation – Support Species Survival in The Wild. Island Press.

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xii. Leadley & Greene (Eds.) (1998). The Darwin Technical Manual for Botanic Gardens. BGCI, London. Available in English, French or Spanish.

xiii Taking into account germination rates, losses during storage and seedling survival rates to maturity.

xiv Taking into account cutting survival rates to maturity (maturity here = mature enough to be suitable stock for vegetative propagation, not necessarily sexual maturity).

xv Hamrick J M et al (1991) Correlations between species traits and allozyme diversity: implications for conservation biology. P75-86 in Falk D and Holsinger K (Eds) Genetics and Conservation of Rare Plants. New York: Oxford University Press, referenced in Schaal B and Leverich WJ (2004) Population Genetic Issues in Ex Situ Plant Conservation. In Guerrant E O *et al* (Eds) Ex Situ Plant Conservation – Supporting Species Survival in The Wild. Island Press.

xvi Falk D A et al (2001) An introduction to restoration genetics. Prepared by the Society for Restoration Ecology, for Plant Conservation Alliance, Bureau of Land Management, US Department of interior,

xvii Buerkle et al (2003) The Origin and Extinction of Species Through Hybridization. In Brigham CA & Schwartz MW (Eds) (2003). Population Viability in Plants – Conservation, Management, and Modeling of Rare Plants.

xviii Oostermeijer JGB (2003) Threats to Rare Plant Persistence. In Brigham CA & Schwartz MW (Eds) (2003). Population Viability in Plants – Conservation, Management, and Modeling of Rare Plants.

xix Wolf DE et al (2001) Predicting the risk of extinction through hybridisation. Conserv Biol 15;1039-1053.

xx A die or coin can be sufficient to make such decisions. The random sample can be across the whole population, or perhaps an equal number within microhabitat types or within sections of a large grid.

xxi Adapted from *A Handbook for Botanic Gardens on the Reintroduction of Plants to the Wild*. BGCI, April 1995.

xxii Ex situ collections are already known to have been the vector of a number of pests and diseases to wild populations.

xxiii The University of Washington College of Forest Resources produces a good summary of smoke-induction techniques at http://depts.washington.edu/propplnt/2003guidelines/group1/Smoke%20Infusion.htm

xxiv http://www.kew.org/msbp/tech/comparative_longevity.pdf

xxv Online information at http://www.aosaseed.com/reference.html

xxvi www.ipgri.cgiar.org/themes/exsitu/seed_compendium.htm

xxvii In Edward O. Guerrant, Kayri Havens, and Mike Maunder (2004) Ex Situ Plant Conservation: Supporting Species Survival in the Wild. (Society for Ecological Restoration International with Island Press).

xxviii Sugii N and Lamoreux C (2004). Tissue Culture as a conservation Method: An Empirical View from Hawaii. In Guerrant E O *et al* (Eds) Ex Situ Plant Conservation – Support Species Survival in The Wild. Island Press.

xxix Husband B C and Campbell LG (2004) *ibid*

xxx Wolf De E et al (2001) Predicting the risk of extinction through hybridisation. *Conservation Biology* 154: 1039-1053. Referenced in Maunder et al (2004) *ibid*.

xxxi 'Cuttings' used broadly to include layering and other vegetative techniques other than *in vitro* propagation.

xxxii Kareiva P (1985) Finding and losing host plants by *Phyllotreta*: patch size and surrounding habitat. Ecology 66:1809-1816.

xxxiii Akeroyd A and Wyse Jackson P (Compilers) (1995) A Handbook for Botanic Gardens on the Reintroduction of Plants to the Wild. Botanic Gardens Conservation International.

xxxiv Leadley E and Greene J (Eds) (1998) Botanic Gardens Conservation International. Chapter 7.

xxxv Monitoring. In Restoring Diversity – Strategies for Reintroduction of Endangered Plants, Falk Donald A et al (Eds) Island Press, Washington DC.

xxxvi Vallee L et al (2004) Guidelines for the Translocation of Threatened Plants in Australia. Australian Network for Plant Conservation, Canberra.

Appendix A

IUCN Criteria for Endangered and Critically Endangered Taxa

CRITICALLY ENDANGERED (CR)
A taxon is Critically Endangered when the best available evidence indicates that it meets any of the following criteria (A to E), and it is therefore considered to be facing an extremely high risk of extinction in the wild:

A. Reduction in population size based on any of the following:

1. An observed, estimated, inferred or suspected population size reduction of =90% over the last 10 years or three generations, whichever is the longer, where the causes of the reduction are clearly reversible AND understood AND ceased, based on (and specifying) any of the following:
(a) direct observation
(b) an index of abundance appropriate to the taxon
(c) a decline in area of occupancy, extent of occurrence and/or quality of habitat
(d) actual or potential levels of exploitation
(e) the effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.

2. An observed, estimated, inferred or suspected population size reduction of =80% over the last 10 years or three generations, whichever is the longer**,** where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible**,** based on (and specifying) any of (a) to (e) under A1.

3. A population size reduction of =80%, projected or suspected to be met within the next 10 years or three generations, whichever is the longer (up to a maximum of 100 years), based on (and specifying) any of (b) to (e) under A1.

4. An observed, estimated, inferred, projected or suspected population size reduction of =80% over any 10 year or three generation period, whichever is longer (up to a maximum of 100 years in the future), where the time period must include both the past and the future, and where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible, based on (and specifying) any of (a) to (e) under A1.

B. Geographic range in the form of either B1 (extent of occurrence) OR B2 (area of occupancy) OR both:

1. Extent of occurrence estimated to be less than 100 km^2, and estimates indicating at least two of a–c:

a. Severely fragmented or known to exist at only a single location.

b. Continuing decline, observed, inferred or projected, in any of the following:
(i) extent of occurrence
(ii) area of occupancy
(iii) area, extent and/or quality of habitat
(iv) number of locations or subpopulations
(v) number of mature individuals.

c. Extreme fluctuations in any of the following:
(i) extent of occurrence
(ii) area of occupancy
(iii) number of locations or subpopulations
(iv) number of mature individuals.

2. Area of occupancy estimated to be less than 10 km^2, and estimates indicating at least two of a–c:

a. Severely fragmented or known to exist at only a single location.

b. Continuing decline, observed, inferred or projected, in any of the following:
(i) extent of occurrence
(ii) area of occupancy
(iii) area, extent and/or quality of habitat
(iv) number of locations or subpopulations
(v) number of mature individuals.

c. Extreme fluctuations in any of the following:
(i) extent of occurrence
(ii) area of occupancy
(iii) number of locations or subpopulations
(iv) number of mature individuals.

C. Population size estimated to number fewer than 250 mature individuals and either:

1. An estimated continuing decline of at least 25% within three years or one generation, whichever is longer, (up to a maximum of 100 years in the future) OR

2. A continuing decline, observed, projected, or inferred, in numbers of mature individuals AND at least one of the following (a–b):

a. Population structure in the form of one of the following:
(i) no subpopulation estimated to contain more than 50 mature individuals, OR
(ii) at least 90% of mature individuals in one subpopulation.

b. Extreme fluctuations in number of mature individuals.

D. Population size estimated to number fewer than 50 mature individuals.

E. Quantitative analysis showing the probability of extinction in the wild is at least 50% within 10 years or three generations, whichever is the longer (up to a maximum of 100 years).

ENDANGERED (EN)

A taxon is Endangered when the best available evidence indicates that it meets any of the following criteria (A to E), and it is therefore considered to be facing a very high risk of extinction in the wild:

A. Reduction in population size based on any of the following:

1. An observed, estimated, inferred or suspected population size reduction of =70% over the last 10 years or three generations, whichever is the longer, where the causes of the reduction are clearly reversible AND understood AND ceased, based on (and specifying) any of the following:

(a) direct observation
(b) an index of abundance appropriate to the taxon
(c) a decline in area of occupancy, extent of occurrence and/or quality of habitat
(d) actual or potential levels of exploitation
(e) the effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.

2. An observed, estimated, inferred or suspected population size reduction

of =50% over the last 10 years or three generations, whichever is the longer**,** where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible, based on (and specifying) any of (a) to (e) under A1.

3. A population size reduction of =50%, projected or suspected to be met within the next 10 years or three generations, whichever is the longer (up to a maximum of 100 years), based on (and specifying) any of (b) to (e) under A1.

4. An observed, estimated, inferred, projected or suspected population size reduction of =50% over any 10 year or three generation period, whichever is longer (up to a maximum of 100 years in the future), where the time period must include both the past and the future, and where the reduction or its causes may not have ceased OR may not be understood OR may not be reversible, based on (and specifying) any of (a) to (e) under A1.

B. Geographic range in the form of either B1 (extent of occurrence) OR B2 (area of occupancy) OR both:

1. Extent of occurrence estimated to be less than 5000 km^2, and estimates indicating at least two of a–c:

a. Severely fragmented or known to exist at no more than five locations.

b. Continuing decline, observed, inferred or projected, in any of the following:
(i) extent of occurrence
(ii) area of occupancy
(iii) area, extent and/or quality of habitat
(iv) number of locations or subpopulations
(v) number of mature individuals.

c. Extreme fluctuations in any of the following:
(i) extent of occurrence
(ii) area of occupancy
(iii) number of locations or subpopulations
(iv) number of mature individuals.

2. Area of occupancy estimated to be less than 500 km^2, and estimates indicating at least two of a–c:
a. Severely fragmented or known to exist at no more than five locations.

b. Continuing decline, observed, inferred or projected, in any of the following:
(i) extent of occurrence
(ii) area of occupancy
(iii) area, extent and/or quality of habitat
(iv) number of locations or subpopulations
(v) number of mature individuals.

c. Extreme fluctuations in any of the following:
(i) extent of occurrence
(ii) area of occupancy
(iii) number of locations or subpopulations
(iv) number of mature individuals.

C. Population size estimated to number fewer than 2500 mature individuals and either:

1. An estimated continuing decline of at least 20% within five years or two generations, whichever is longer, (up to a maximum of 100 years in the future) OR

2. A continuing decline, observed, projected, or inferred, in numbers of mature individuals AND at least one of the following (a–b):

a. Population structure in the form of one of the following:
(i) no subpopulation estimated to contain more than 250 mature individuals, OR
(ii) at least 95% of mature individuals in one subpopulation.

b. Extreme fluctuations in number of mature individuals.

D. Population size estimated to number fewer than 250 mature individuals.

E. Quantitative analysis showing the probability of extinction in the wild is at least 20% within 20 years or five generations, whichever is the longer (up to a maximum of 100 years).

Appendix B

IUCN SSC Re-introduction Guidelines: Guideline 4a (v) Availability of Suitable Release Stock

(v) Availability of suitable release stock

* It is desirable that source animals come from wild populations. If there is a choice of wild populations to supply founder stock for translocation, the source population should ideally be closely related genetically to the original native stock and show similar ecological characteristics (morphology, physiology, behaviour, habitat preference) to the original sub-population.
* Removal of individuals for re-introduction must not endanger the captive stock population or the wild source population. Stock must be guaranteed available on a regular and predictable basis, meeting specifications of the project protocol.
* Individuals should only be removed from a wild population after the effects of translocation on the donor population have been assessed, and after it is guaranteed that these effects will not be negative.
* If captive or artificially propagated stock is to be used, it must be from a population which has been soundly managed both demographically and genetically, according to the principles of contemporary conservation biology.
* Re-introductions should not be carried out merely because captive stocks exist, nor solely as a means of disposing of surplus stock.
* Prospective release stock, including stock that is a gift between governments, must be subjected to a thorough veterinary screening process before shipment from original source. Any animals found to be infected or which test positive for non-endemic or contagious pathogens with a potential impact on population levels, must be removed from the consignment, and the uninfected, negative remainder must be placed in strict quarantine for a suitable period before retest. If clear after retesting, the animals may be placed for shipment.
* Since infection with serious disease can be acquired during shipment, especially if this is intercontinental, great care must be taken to minimize this risk.
* Stock must meet all health regulations prescribed by the veterinary authorities of the recipient country and adequate provisions must be made for quarantine if necessary.

Appendix C

Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act. October 20, 2000.

Summary

This policy, published jointly by the Fish and Wildlife Service (FWS) and the National Marine Fisheries Service (NMFS), addresses the role of controlled propagation in the conservation and recovery of species listed as endangered or threatened under the Endangered Species Act. The policy provides guidance and establishes consistency for use of controlled propagation as a component of a listed species recovery strategy. Appropriate uses of controlled propagation include supporting recovery related research, maintaining refugia populations, providing plants or animals for reintroduction or augmentation of existing populations, and conserving species or populations at risk of imminent extinction or extirpation.

This policy supports and promotes coordination between various phases of controlled propagation efforts such as propagation technology development, propagation for release, population augmentation, reintroduction, and monitoring. This policy will also contribute to the efficient use of funding resources.

Guidance is provided regarding the use of controlled propagation for:

* Preventing the extinction of listed species, subspecies, or populations;
* Recovery-oriented scientific research, including, but not restricted to, developing propagation methods and technology, and other actions that are expected to result in a net benefit to the listed taxon. Use of surrogates, while applicable to the recovery of listed species, is exempt from the requirements of this policy;
* Maintaining genetic vigor and demographic diversity of listed species, subspecies, or populations;
* Maintaining refugia populations for nearly extinct animals or plants on a temporary basis until threats to a listed species' habitat are alleviated, or necessary habitat modifications are completed, or when potentially catastrophic events occur (e.g.,
* Chemical spills, severe storms, fires, flooding);
* Providing individuals for establishing new, self-sustaining populations necessary for recovery of the listed species; and
* Supplementing or enhancing extant populations to facilitate recovery of the listed species.

Scope

This policy applies to all pertinent organizational elements of both Services, notwithstanding those differences in administrative procedures and policies as noted. Exceptions to this policy appear in section F. This policy pertains to all efforts Requiring permits under 50 CFR 17 subparts C and D, funded, authorized, or carried out by us that are conducted to propagate threatened or endangered species by:

* Establishing or maintaining refugia populations;
* Producing individuals for research and technology development needs;
* Producing individuals for supplementing extant populations; and

* Producing individuals for reintroduction to suitable habitat within the species' historic range.

Need

The controlled propagation of animals and plants in certain situations is an essential tool for the conservation and recovery of listed species. In the past, we have used controlled propagation to reverse population declines and to successfully return listed species to suitable habitat in the wild.

Though controlled propagation has a supportive role in the recovery of some listed species, the intent of the Act is "to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved." Controlled propagation is not a substitute for addressing factors responsible for an endangered or threatened species' decline. Therefore, our first priority is to recover wild populations in their natural habitat wherever possible, without resorting to the use of controlled propagation. This position is fully consistent with the Act.

We recognize that genetic and ecological risks may be associated with introducing to the wild, animals and plants bred and reared in a controlled environment. When considering controlled propagation as a recovery option, the potential benefits and risks must be assessed and alternatives requiring less intervention objectively Evaluated. If controlled propagation is identified as an appropriate strategy for the recovery of a listed species, it must be conducted in a manner that will, to the maximum extent possible, preserve the genetic and ecological distinctiveness of the listed species and minimize risks to existing wild populations.

We recognize that for many species, information available for detailed genetics conservation management or assessment of risks associated with reintroduction may be insufficient. Therefore, this policy does not specifically require written genetic management plans and ecological risk assessments to initiate or support controlled propagation programs. Additionally, acute conservation needs may legitimately outweigh delays that would be incurred by such a requirement. However, where sufficient biological and environmental information exists, and where conservation activities would not be unduly constrained, a formal assessment of ecological and genetic risks is strongly encouraged. Risks that must be evaluated in the planning of controlled propagation programs include the following specific examples:

* Removal of natural parental stock that may result in an increased risk of extinction by reducing the abundance of wild individuals and reducing genetic variability within naturally occurring populations;
* Equipment failures, human error, disease, and other potential catastrophic events that may cause the loss of some or all of the population being held or maintained in captivity or cultivation;
* The potential for an increased level of inbreeding or other adverse genetic effects within populations that may result from the enhancement of only a portion of the gene pool;
* Potential erosion of genetic differences between populations as a result of mixed stock transfers or supplementation;
* Exposure to novel selection regimes in controlled environments that may diminish a listed species' natural capacity to survive and reproduce in the wild;
* Genetic introgression, which may diminish local adaptations of the naturally occurring population;

* Increased predation, competition for food, space, mates, or other factors that may displace naturally occurring individuals, or interfere with foraging, migratory, reproductive, or other essential behaviors; and
* Disease transmission.

Controlled propagation programs must be undertaken in a manner that minimizes potentially adverse impacts to existing wild populations of listed species, and we must conduct controlled propagation programs in a manner that avoids additional listing actions.

The Policy

Our policy is that the controlled propagation of threatened and endangered species will be:

1. Used as a recovery strategy only when other measures employed to maintain or improve a listed species' status in the wild have failed, are determined to be likely to fail, are shown to be ineffective in overcoming extant factors limiting recovery, or would be insufficient to achieve full recovery. All reasonable effort should be made to accomplish conservation measures that enable a listed species to recover in the wild, with or without intervention (e.g., artificial cavity provisioning), prior to implementing controlled propagation for reintroduction or supplementation.

2. Coordinated with conservation actions and other recovery measures, as appropriate or specified in recovery plans, that will contribute to, or otherwise support, the provision of secure and suitable habitat. Controlled propagation programs intended for reintroduction or augmentation must be coordinated with habitat management, restoration, and other species' recovery efforts.

3. Based on the specific recommendations of recovery strategies identified in approved recovery plans or supplements to approved recovery plans whenever practical. The recovery plan, in addressing controlled propagation, should clearly identify the necessity and role of this activity as a recovery strategy.

4. Based on specific consideration of the potential ecological and genetic effects of the removal of individuals for controlled propagation purposes on wild populations and the potential effects of introductions of artificially bred animals or plants on the receiving population and other resident species. Assessments of potential risks and benefits will be addressed, as required, through sections 7 and 10 of the Act and the National Environmental Policy Act (NEPA, 42 U.S.C. 4332) for proposed controlled propagation actions.

5. Based on sound scientific principles to conserve genetic variation and species integrity. Intercrossing will not be considered for use in controlled propagation programs unless recommended in an approved recovery plan; supported in an approved genetic management plan (if information is available to develop such a plan, and which may or may not be part of an approved recovery plan); implemented in a scientifically controlled and approved manner; and undertaken to compensate for a loss of genetic viability in listed taxa that have been genetically isolated in the wild as a result of human activity. Use of intercross individuals for species conservation will require the approval of the FWS Director or that of the NMFS Assistant

Administrator, in accordance with all applicable policies.

6. Preceded, when practical, by the development of a genetics management plan based on accepted scientific principles and procedures. Controlled propagation protocols will follow accepted standards such as those employed by the American Zoo and Aquarium Association (AZA), the Center for Plant Conservation (CPC), and Federal agency protocols such as fish management guidelines to the extent practical.

All efforts will be made by us and our cooperators to ensure that the genetic makeup of propagated individuals is representative of that in free-ranging populations and that propagated individuals are behaviorally and physiologically suitable for introduction. Determination of biological "suitability" may include, but should not necessarily be limited to, analysis of geomorphological similarities of habitat, genetic similarity, phenotypic characteristics, stock histories, habitat use, and other ecological, biological, and behavioral indicators. All controlled propagation programs will address the issue of disposition of individuals found to be:

(a) Unfit for introduction to the wild;
(b) Unfit to serve as broodstock;
(c) Surplus to program needs; or
(d) Surplus to the recovery needs for the species (e.g., to preclude genetic and ecological swamping).

Controlled propagation activities should not be initiated without including consideration of these issues and obtaining required permits and other authorizations as necessary. Disposition of individuals surplus to program needs may include use for research or other appropriate purposes.

Programs involving the controlled propagation of listed species for research purposes identified in final recovery plans and in which progeny will not be reintroduced to the wild are exempt from this policy. Examples of exempt actions include research involving the determination of germination rates in plants and spawning success rates in fish. This exemption does not extend to the need for these activities to comply with any other applicable Federal or State permitting or regulatory requirements.

7. Conducted in a manner that takes all known precautions to prohibit the potential introduction or spread of diseases and parasites into controlled environments or suitable habitat.

8. Conducted in a manner that will prevent the escape or accidental introduction of individuals outside their historic range.

9. Conducted, when feasible, at more than one location in order to reduce the potential for catastrophic loss at a single facility when a substantial fraction of a species or important population segment is brought into captivity.

10. Coordinated, as appropriate, with organizations and qualified individuals both within and outside our agencies. We will cooperate with other Federal agencies and State, Tribal, and local governments.

11. Conducted in a manner that will meet our information needs and that will be in accordance with accepted protocols and standards. In the case of listed species for

which traditional studbooks or registrations are not practical, records of eggs, larvae, or other life-stages will be maintained.

12. With limited exceptions, implemented only after a commitment to funding is secured.

13. Prior to releases of propagated individuals, tied to development of a reintroduction plan, unless this information is already contained in an approved recovery plan, species survival plan, or equivalent document that has received the approval of the appropriate Service. Controlled propagation and reintroduction plans will identify measurable objectives and milestones for the proposed propagation and reintroduction effort. The controlled propagation and reintroduction plan should be based on strategies identified in the approved recovery plan. It should include protocols for health management, disease screening and disease-free certification, monitoring and evaluation of genetic, demographic, life-history, phenotypic, and behavioral characteristics, data collection, recordkeeping, and reporting as appropriate. On implementation, periodic evaluations must be made to assess project progress and consider new scientific information and the status of habitat conservation efforts.

14. Conducted in accordance with the regulations implementing the Endangered Species Act, Marine Mammal Protection Act, Animal Welfare Act, Lacey Act, Fish and Wildlife Act of 1956, and the Services' procedures relative to NEPA.

Appendix D

Avoiding Hybridisation

Proposed guidelines to reduce:

* spontaneous hybridisation within ex situ plant collections
* the release of potential invasive hybrid taxa
* hybridisation in management of threatened taxa.

From Maunder M et al (2004) Hybridization in Ex Situ Plant Collections. In Guerrant E O *et al* (Eds) Ex Situ Plant Conservation – Support Species Survival in The Wild. Island Press.

Collection Management

* Enact policies to minimise undesirable spontaneous hybridisation in terms of what species are grown and how collections are designed, laid out, and horticuturally managed.
* Plan collections to minimise hybridisation by planting congenerics as far apart as possible.
* Manage collections to reduce the production or persistence of hybrids (e.g. by effective weed control and dead-heading of seeding plants)
* Where hybridisation is anticipated or expected, bag inflorescences or remove them from congenerics around plants used for seed production, and produce seed using controlled pollination.
* Physically separate conservation and horticultural display facilities.
* Avoid the use or promotion of geographically or taxonomically themed collections as conservation resources, use them as educational displays only, and practice strict prevention of seed production and distribution.
* Avoid planting single-genus collections near important wild populations of congenerics. Particular concern should be given to the planting of hybrids and cultivars derived from indigenous and local wild species.
* Identify high-risk plant groups in terms of hybridisation and invasive tendencies and avoid cultivating species from this group.

Material Exchange

* Tailor acquisition, exchange, distribution, and release policies to minimise unnecessary escape or release of potentially invasive species, and warn recipients about risks associated with species introductions, including hybridisation.

Impact Assessment and Knowledge

* Assess and monitor the likely and actual impacts of *ex situ* collections on adjacent natural or semi-natural habitats in collaboration wit management authorities for nearby habitats and threatened species.

Training

* Make all horticultural and scientific staff associated with *ex situ* collections aware of the risks and possible consequences of hybridisation within or derived from *ex situ* collections.

Education

* Use living collections to demonstrate and interpret what hybrids are, how pollen flows, and the ecological and economic dangers of invasive species.

Species Management

Propagation of threatened species must aim to minimise the negative impacts of spontaneous hybridisation by:

* Establishing the taxonomic status and hybridity of the threatened taxon (i.e. is the target taxon a natural hybrid or part of a hybrid swarm or complex of infertile taxa?).

* Establishing the susceptibility of the taxon to hybridisation. Does the taxonomic, biosystematic, or horticultural literature indicate that this taxon (or genus) is susceptible to hybridisation in the wild and in cultivation?

* Clarifying the conservation objectives. Does the recovery plan specify the role of the *ex situ* facility in terms of necessary propagules types (seed or vegetative) and genetic status? Where possible, use vegetative propagation for high-risk taxa.

* Dedicating isolated propagation facilities for the plant. Produce seed under controlled and insect- and pollen-screened conditions.

* Removing or reducing any congenerics or sister genera in the immediate environs (wild and cultivated) that could hybridise with the target taxon.

* Assessing origins of all founder stocks, with particular attention to seed-derived founders from unmanaged *ex situ* collections where hybridisation may have occurred. Genetic screening is recommended in this situation.

* Minimising the number of reproductive generations and using seed storage to store propagules. Where appropriate, use clonal or vegetative duplication or propagation.

Research

* Liaise with researchers to promote and stimulate research studies of hybrid problems, invasive origins, and hazards and basic reproductive and population biology of threatened taxa.

Appendix E

IUCN Technical Guidelines on the Management of Ex-situ Populations for Conservation

Approved at the 14th Meeting of the Programme Committee of Council, Gland Switzerland, 10 December 2002.

PREAMBLE

IUCN affirms that a goal of conservation is the maintenance of existing genetic diversity and viable populations of all taxa in the wild in order to maintain biological interactions, ecological processes and function. Conservation managers and decision-makers should adopt a realistic and integrated approach to conservation implementation. The threats to biodiversity in situ continue to expand, and taxa have to survive in increasingly human-modified environments. Threats, which include habitat loss, climate change, unsustainable use, and invasive and pathogenic organisms, can be difficult to control. The reality of the current situation is that it will not be possible to ensure the survival of an increasing number of threatened taxa without effectively using a diverse range of complementary conservation approaches and techniques including, for some taxa, increasing the role and practical use of ex situ techniques.

If the decision to bring a taxon under ex situ management is left until extinction is imminent, it is frequently too late to effectively implement, thus risking permanent loss of the taxon. Moreover, ex situ conservation should be considered as a tool to ensure the survival of the wild population. Ex situ management should be considered only as an alternative to the imperative of in situ management in exceptional circumstances, and effective integration between in situ and ex situ approaches should be sought wherever possible.

The decision to implement an ex situ conservation programme as part of a formalised conservation management or recovery plan and the specific design of and prescription for such an ex situ programme will depend on the taxon's circumstances and conservation needs. A taxon-specific conservation plan may involve a range of ex situ objectives, including short-, medium- and long-term maintenance of ex situ stocks. This can utilise a variety of techniques including reproduction propagation, germplasm banking, applied research, reinforcement of existing populations and re-introduction into the wild or controlled environments. The objectives and overall purpose should be clearly stated and agreed among organisations participating in the programme, and other relevant stakeholders including landowners and users of the taxon involved. In order to maximise their full potential in conservation, ex situ facilities and their co-operative networks should adopt the guidelines defined by the Convention on Biological Diversity (CBD), the International Agenda for Botanic Gardens in Conservation, Center for Plant Conservation and the World Zoo Conservation Strategy, along with other guidelines, strategies, and relevant legislative requirements at national and regional levels. IUCN recognizes the considerable set of resources committed worldwide to ex situ conservation by the world's zoological and botanical gardens, gene banks and other ex situ facilities. The effective utilisation of these resources represents an essential component of conservation strategies at all levels.

VISION

To maintain present biodiversity levels through all available and effective means including, where appropriate, ex situ propagation, translocation and other ex situ methodologies.

GOAL

Those responsible for managing ex situ plant and animal populations and facilities will use all resources and means at their disposal to maximise the conservation and utilitarian values of these populations, including:

1) increasing public and political awareness and understanding of important conservation issues and the significance of extinction;
2) co-ordinated genetic and demographic population management of threatened taxa;
3) re-introduction and support to wild populations;
4) habitat restoration and management;
5) long-term gene and biomaterial banking;
6) institutional strengthening and professional capacity building;
7) appropriate benefit sharing;
8) research on biological and ecological questions relevant to in situ conservation; and
9) fundraising to support all of the above.

Ex situ agencies and institutions must follow national and international obligations with regard to access and benefit sharing (as outlined in the CBD) and other legally binding instruments such as CITES, to ensure full collaboration with all range States. Priority should be given to the ex situ management of threatened taxa (according to the latest IUCN Red List Categories) and threatened populations of economic or social/cultural importance. Ex situ programmes are often best situated close to or within the ecogeographic range of the target taxa and where possible within the range State. Nevertheless a role for international and extra regional support for ex situ conservation is also recognised. The option of locating the ex situ programme outside the taxa's natural range should be considered if the taxa is threatened by natural catastrophes, political and social disruptions, or if further germplasm banking, propagation, research, isolation or reintroduction facilities are required and cannot be feasibly established. In all cases, ex situ populations should be managed in ways that minimize the loss of capacity for expression of natural behaviours and loss of ability to later again thrive in natural habitats.

TECHNICAL GUIDELINES

The basis for responsible ex situ population management in support of conservation is founded on benefits for both threatened taxa and associated habitats.

* The primary objective of maintaining ex situ populations is to help support the conservation of a threatened taxon, its genetic diversity, and its habitat. Ex situ programmes should give added value to other complementary programmes for conservation.

Although there will be taxa-specific exceptions due to unique life histories, the decision to initiate ex situ programmes should be based on one or more of the appropriate IUCN Red List Criteria, including:

1. When the taxa/population is prone to effects of human activities or stochastic events or

2. When the taxa/population is likely to become Critically Endangered, Extinct in the Wild, or Extinct in a very short time. Additional criteria may need to be considered in some cases where taxa or populations of cultural importance, and significant economic or scientific importance, are threatened. All Critically Endangered and Extinct in the Wild taxa should be subject to ex situ management to ensure recovery of wild populations.

* Ex situ conservation should be initiated only when an understanding of the target taxon's biology and ex situ management and storage needs are at a level where there is a reasonable probability that successful enhancement of species conservation can be achieved; or where the development of such protocols could be achieved within the time frame of the taxon's required conservation management, ideally before the taxa becomes threatened in the wild. Ex situ institutions are strongly urged to develop ex situ protocols prior to any forthcoming ex situ management. Consideration must be given to institutional viability before embarking on a long term ex situ project.
* For those threatened taxa for which husbandry and/or cultivation protocols do not exist, surrogates of closely related taxa can serve important functions, for example in research and the development of protocols, conservation biology research, staff training, public education and fundraising.
* While some ex situ populations may have been established prior to the ratification of the CBD, all ex situ and in situ populations should be managed in an integrated, multidisciplinary manner, and where possible, in accordance with the principles and provisions of the CBD.
* Extreme and desperate situations, where taxa/populations are in imminent risk of extinction, must be dealt with on an emergency basis. This action must be implemented with the full consent and support of the range State.
* All ex situ populations must be managed so as to reduce risk of loss through natural catastrophe, disease or political upheaval. Safeguards include effective quarantine procedures, disease and pathogen monitoring, and duplication of stored germplasm samples in different locations and provision of emergency power supplies to support collection needs (e.g. climate control for long term germplasm repositories).
* All ex situ populations should be managed so as to reduce the risk of invasive escape from propagation, display and research facilities. Taxa should be assessed as to their invasive potential and appropriate controls taken to avoid escape and subsequent naturalisation.
* The management of ex situ populations must minimise any deleterious effects of ex situ management, such as loss of genetic diversity, artificial selection, pathogen transfer and hybridisation, in the interest of maintaining the genetic integrity and viability of such material. Particular attention should be paid to initial sampling techniques, which should be designed to capture as much wild genetic variability as practicable. Ex situ practitioners should adhere to,

and further develop, any taxon- or region-specific record keeping and genetic management guidelines produced by ex situ management agencies.

* Those responsible for managing ex situ populations and facilities should seek both to increase public awareness, concern and support for biodiversity, and to support the implementation of conservation management, through education, fundraising and professional capacity building programmes, and by supporting direct action in situ.
* Where appropriate, data and the results of research derived from ex situ collections and ex situ methodologies should be made freely available to ongoing in-country management programmes concerned with supporting conservation of in situ populations, their habitats, and the ecosystems and landscapes in which they occur .

NB. Ex situ conservation is defined here, as in the CBD, as "the conservation of components of biological diversity outside their natural habitats". Ex situ collections include whole plant or animal collections, zoological parks and botanic gardens, wildlife research facilities, and germplasm collections of wild and domesticated taxa (zygotes, gametes and somatic tissue).