Guidelines for genetic monitoring of translocated plant populations

Fabienne Van Rossum[1,2], Olivier J. Hardy[3]

[1]Meise Botanic Garden, Nieuwelaan 38, 1860 Meise, Belgium

[2] Service général de l'Enseignement supérieur et de la Recherche scientifique, Fédération Wallonie-Bruxelles, rue A. Lavallée 1, 1080 Brussels, Belgium

[3]Unit of Evolutionary Biology and Ecology, Université Libre de Bruxelles, Avenue F.D. Roosevelt 50, CP 160/12, 1050 Brussels, Belgium

Corresponding author: Fabienne Van Rossum; Meise Botanic Garden, Nieuwelaan 38, 1860 Meise, Belgium; e-mail: fabienne.vanrossum@botanicgardenmeise.be

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Abstract

Plant translocation is a useful tool for implementing assisted gene flow in recovery plans of critically endangered plant species. Although it helps to restore genetically viable populations, it is not devoid of genetic risks, such as poor adaptation of transplants and outbreeding depression in the hybrid progeny, which may have negative consequences in terms of demographic growth and plant fitness. Hence, a follow-up genetic monitoring should evaluate whether the translocated populations are genetically viable and self-sustaining in the short and long term. We also need to identify the causes of failure to adjust management responses. Here we show how molecular markers and fitness-related quantitative traits can evaluate the following: (1) genetic diversity enhancement; (2) increased plant fitness; (3) long-term trends. The proposed guidelines, illustrated with studies from the literature, will help the practitioners to find the appropriate genetic survey methods, so that management practices can better integrate evolutionary processes.

Introduction

Plant translocations consist of intentionally introducing living organisms from one area to another, usually as spores, seeds or plug plants (Menges 2008; Weeks et al. 2011). Translocation is a useful tool for implementing assisted gene flow in recovery plans of critically endangered plant species: bringing new, genetically diverse material will allow for genetic restoration and/or rescue. Genetic restoration refers to the recovery of genetic diversity and evolutionary resilience of the population (Weeks et al. 2011), while genetic rescue aims at counteracting the expression of genetic load (deleterious genes) to improve plant fitness (Bell et al. 2019). Accordingly, plant translocations can be used for population

reinforcements, reintroductions and introductions. Reinforcements (also called augmentations) consist of translocating additional plants in small and isolated endangered populations (Betz et al. 2013; Ottewell et al. 2016), allowing for genetic restoration and/or rescue when populations are depauperate and/or inbred (Weeks et al. 2011; Zavodna et al. 2015). However, when populations have gone extinct, and when we cannot count on a persistent seed bank in the soil or on seed recolonization from neighboring sites, it is necessary to recreate new populations after suitable habitat restoration by reintroducing plants (Menges 2008; Weeks et al. 2011). If the original sites do not exist anymore or cannot be restored to achieve suitable habitat conditions, introductions may be considered in other sites, possibly after an ecological restoration phase (Colas et al. 2008).

Although plant translocations aim at restoring genetically viable populations, they may be associated with genetic risks, which may have negative consequences in terms of demographic growth and plant fitness or regarding the conservation of local genetic diversity. When local populations are too depauperate or inbred, the translocation of a highly diverse genetic pool, possibly using nonlocal or/and mixed source populations can maximize evolutionary resilience, counteract the detrimental effects of inbreeding depression, and increase the number of compatible mates (Weeks et al. 2011; Maschinski et al. 2013; Zavodna et al. 2015). However, the introduced and local genotypes may be genetically very different from each other, so that the new transplants and their progeny may be poorly adapted to the translocated sites, and local genetic variability may be lost. Outbreeding depression, i.e. the lower fitness of hybrid offspring, may be expressed in terms of seed germination, plant growth and survival because of the breakdown of positive epistatic interactions in local co-adapted gene complexes. Hybrid generations may also display

intermediate phenotypes that are less adapted than the parental ones (Edmands 2007; Frankham et al. 2011; Weeks et al. 2011). Nevertheless, the risk of outbreeding depression after a few generations of cross mating might be compensated by an increase in plant fitness expected with heterosis (Willi et al. 2007). Moreover, natural selection will favor the best adapted genotypes in the long term (Sgrò et al. 2011).

To maximize the ability to recover demographically sustainable and evolutionary resilient populations, the following should occur prior to implementation of translocations: (1) estimate the genetic and demographic status of the populations to reinforce; (2) carefully select appropriate target sites and source populations for translocation; (3) design appropriate plant propagation protocols; and (4) carefully prepare the transplantation in the field (Menges 2008; IUCN/SSC 2013; Basey et al. 2015; Godefroid et al. 2016; Ottewell et al. 2016; Maschinski & Albrecht 2017; Commander et al. 2018). Based on these prior evaluations, it is important that the recovery plan first determines what are the goals of the translocation: restoration of demographically viable populations, genetic restoration / rescue of genetically depauperate / inbred populations and/or creation of additional populations (Weeks et al. 2011). Furthermore, to assess whether the targeted goals are achieved, the recovery plan must select appropriate success criteria, i.e. measurable population parameters that estimate whether translocation improves long-term population viability (Menges 2008). After the translocation, it is necessary to implement follow-up demographic and genetic monitoring to evaluate these success criteria. We also need to identify the causes of failure to adjust management responses (Schwartz et al. 2007; Menges 2008; Godefroid & Van Rossum 2018).

Demographic monitoring is very useful to assess population growth by recruitment, plant survival and reproductive success (Godefroid et al. 2011; Commander et al. 2018; Albrecht et al. 2019; Fenu et al. 2019). However, it cannot give insights into the evolutionary potential of translocated populations nor into the detailed reproductive processes occurring after translocation, which are key determinants of the success or failure of the translocation (Schwartz et al. 2007; Van Rossum et al. 2020). For instance, genetic monitoring allows quantifying gene flow, the degree of admixture between local and introduced gene pools, and inbreeding or outbreeding depression. Moreover, many perennial plant species can also propagate asexually, so that population census size may increase while genotypic diversity remains low or decays (Menges 2008; Becheler et al. 2017; Van Rossum & Raspé 2018). Recruitment from sexual reproduction, an important indicator of translocation success (Menges 2008; Albrecht et al. 2019), can be distinguished from clonal propagation using molecular markers (Peakall & Smouse 2006), not by demographic measures. Therefore, genetic monitoring is an important tool for evaluating translocated population sustainability.

It is only recently that recovery plans have begun to involve thorough genetic monitoring of plant populations that have been restored or rescued through reinforcements or (re)introductions (e.g. Zavodna et al. 2015; Rodríguez-Rodríguez et al. 2018). Practical information and framework for implementing genetic surveys of threatened animal and plant species are available (e.g. Schwartz et al. 2007; Menges 2008; Flanagan et al. 2018; Godefroid & Van Rossum 2018). However, they are still incomplete for translocated plant populations or are lost in more general reviews. Plants have particular characteristics compared to most animals, such as the inability to escape unsuitable habitat conditions, gene dispersal mediated by pollen and seeds, and often the ability to propagate asexually. Plant

translocations also show some specificities, such as the need to transplant a large number of individuals, often of a single founder stage (seeds or juveniles), which creates a population with an even age or stage structure (Godefroid et al. 2011; Maschinski & Albrecht 2017). When transplants are previously grown in *ex situ* conditions, selective conditions may differ from the field, possibly leading to differential survival and genetic variation (Alonso et al. 2014; Basey et al. 2015; St. Clair et al. 2020). Therefore, guidelines for monitoring translocations of plant species somewhat differ from animal species. In the present paper, we review the existing literature on genetic monitoring of translocated plant populations. We propose a series of practical guidelines for the genetic monitoring necessary to evaluate the success of plant translocations, including methodological aspects and genetic data analyses, organized according to the goal of the translocation, the sources used, and the possible questions to address (success criteria).

Evaluating the success of plant translocation by genetic monitoring

Genetic monitoring evaluates whether plant translocation has been successful, so whether it has led to genetically viable and self-sustaining populations, and identifies the causes of failure. Accordingly, different questions can be addressed to evaluate translocation success in the short term (over a few generations) and in the long term, using molecular markers and fitness traits. These questions will depend on the predefined goals of the translocation, i.e. whether the translocation consists of a reinforcement of depauperate and/or inbred populations or a (re)introduction, and on the sources used for producing transplants (local, nonlocal, mixed) (Figure 1):

Firstly, is genetic diversity sufficient for ensuring population evolutionary resilience? This means that genetic diversity has been enhanced and/or is higher or comparable to healthy

natural (reference) populations. The extent of genetic differentiation between generations and between translocated populations is expected to be lower or comparable to reference populations. The associated criteria of successful genetic restoration are:

- There is contemporary gene flow, and gene dispersal is sufficient within the translocated populations to maintain low inbreeding levels.
- For clonally propagating species, sexual reproduction, not only clonal propagation, contributes to recruitment.
- In case of mixed and/or nonlocal source populations, the progeny results from admixture between sources, and local genetic diversity is represented in the offspring.

Secondly, is adaptive variation sufficient for ensuring population growth and survival? This means that fitness of plants suffering from detrimental genetic effects has been increased through the introduction of new genetic variation and/or through heterosis. Inbreeding depression has been alleviated. In case of mixed or nonlocal sources, local adaptation has been maintained, transplants have no maladaptation issues, and there is no outbreeding depression in the cross progeny.

- Finally, in the long term, do the recovered populations show effective population size high enough to be viable?

Guidelines for genetic monitoring of translocated plant populations

1. Sampling methodology for assessing translocation success

To evaluate translocation success, the first step is to design an appropriate sampling methodology and choose the appropriate markers (Figure 1). Specific expertise and equipment may be required, so collaborative work between conservation practitioners and evolutionary geneticists is certainly to be encouraged.

1.A. Field sampling over several generations

Genetic data should be obtained for the populations used as source for translocation, and for several generations of the translocated populations (transplants and newly established individuals and their seed progeny). For some species, the long recruitment time lags represent a constraint for monitoring new generations (Bowles et al. 2015; Fotinos et al. 2015; Rodríguez-Rodríguez et al. 2018). Natural healthy populations may serve as reference populations (Menges 2008). Sample sizes –number of individuals and number of seeds per maternal plant for examining seed progeny– depend on the addressed question(s) and on the analyses to be performed (e.g. Basey et al. 2015; Godefroid & Van Rossum 2018). Mapping individuals in the field can be required for estimating pollen and seed dispersal distances (see section 3).

1.B. Which molecular markers?

Suitable molecular markers (DNA fragments) for quantifying genetic diversity and structure should be highly polymorphic (variable) such as microsatellites, or numerous (many loci) such as single-nucleotide polymorphisms (SNPs). They should be preferably co-dominant, meaning that distinct alleles (variants for a gene) can be distinguished in heterozygotes (Peakall & Smouse 2006; Flanagan & Jones 2019). Dominant markers, such as amplified fragment-length polymorphisms (AFLPs) or inter simple sequence repeats (ISSRs), where heterozygotes cannot be distinguished from homozygotes for the dominant allele, can also be useful but such binary data (presence-absence scores) are not suitable to infer inbreeding, a key factor for assessing the success of the translocations (Schwartz et al. 2007; Weeks et al. 2011). Investing in laboratory equipment (e.g. extraction tools, PCR devices, and a capillary

sequencer) may be very expensive (several tens of thousands US$), so that it is interesting to outsource the molecular analyses to external firms or academic laboratories.

The variation in molecular markers such as microsatellites or AFLPs are usually considered to represent neutral genetic processes, thus not related to fitness, although any marker can potentially be linked to a selected gene. Therefore, they are very useful to quantify overall genetic diversity and differentiation, gene flow and infer admixture or recruitment mode. However, they may fail in providing insight into inbreeding or outbreeding depression and adaptation-related genetic diversity (Leinonen et al. 2008). The genomic approach using next generation sequencing technology, by screening large regions of the genome and developing a large number of markers, is a promising tool in facilitating the identification of putatively adaptive or detrimental genes and of the breakdown of co-adapted gene complexes (Angeloni et al. 2012; Benestan et al. 2016; Flanagan et al. 2018). Most molecular data analyses can be performed using various freely available software (Appendix S1).

1.C. Which fitness-related phenotypic characters?

Quantitative genetics, which is based on continuously varying phenotypic character measurements (Table 1), especially in standardized environmental conditions, can give insight into the genetic variability of traits under selection, and into heterosis and inbreeding/outbreeding depression (Edmands 2007; Sgrò et al. 2011; Zavodna et al. 2015; Barmentlo et al. 2018). Getting enough data for statistically sound analyses can be time-consuming, but usually does not require buying expensive equipment. A lower fitness expressed through a reduced growth, seed abortion, seedling chlorosis and/or low pollen viability may indicate that the individuals suffer from inbreeding or outbreeding depression

(Edmands 2007; Godefroid et al. 2016). Higher plant fitness expressed through higher seed weight, germination rate, growth rate and/or reproductive success may indicate heterosis and genetic rescue or local adaptation if it only concerns local genotypes (Willi et al. 2007; Bell et al. 2019).

1.D. Population demographic and environmental data

The sites targeted for plant translocation may vary in ecological conditions (e.g. in vegetation composition, edaphic conditions, management interventions, competition with other species, herbivory pressure). Ecological conditions, such as climatic conditions and disturbance regimes related to management interventions, may also vary from year to year (Menges 2008; Reckinger et al. 2010; Albrecht & Long 2019). The number of sown seeds or translocated plants and demographic dynamics (survival, flowering and recruitment) may also vary among translocated sites (Colas et al. 2008; Fant et al. 2013; Bowles et al. 2015; Fenu et al. 2019). Therefore, these data should be integrated in the genetic data analyses to disentangle environmental and population demographic effects from genetic restoration and rescue effects (Menges 2008; Godefroid & Van Rossum 2018).

2. Changes in genetic diversity and differentiation

Genetic monitoring should assess whether genetic diversity (on which evolutionary resilience depends) has increased if translocation is a reinforcement, or are comparable to reference populations in a (re)introduction. To control for genetic drift and inbreeding, it is important to verify that the genetic restoration is maintained across generations and whether genetic differentiation within and between translocated populations are low or comparable to reference populations.

2.A. Changes in genetic diversity and differentiation in practice

We have found 16 studies on plant translocations implementing genetic monitoring of translocated plant populations (Table 2), a low number compared to the numerous demographic studies performed (e.g. Godefroid et al. 2011; Albrecht et al. 2019). A majority of studies found that genetic diversity was higher in reinforced populations and higher or similar in reintroduced populations compared with natural populations. Genetic differentiation was low to moderate in the new generations or between populations. In these studies, success was attributed to the large number of transplants planted at high density, and/or the mixing of multiple, sometimes nonlocal, seed sources (Ritchie & Krauss 2012; Fant et al. 2013; Alonso et al. 2014; Zavodna et al. 2015; St. Clair et al. 2020). When a small number of individuals were translocated or when there was a high mortality of the transplants and reduced flowering rate, evidence of inbreeding appeared in subsequent generations (Krauss et al. 2002; Aavik et al. 2012; Fant et al. 2013; Fotinos et al. 2015). The small population sizes reported for many restored populations (Table 2) may challenge their long-term viability. Transplant survival, seed germination and establishment of new recruits may be compromised by poor habitat suitability or by a lack of follow-up habitat management (Godefroid et al. 2011; Albrecht & Long 2019). Pre- and post-translocation management interventions in the sites of translocation have favored genetic restoration and recruitment for *Pulsatilla vulgaris* (Betz et al. 2013) and *Arnica montana* (Van Rossum et al. 2020).

2.B. Guidelines for assessing changes in genetic diversity and differentiation

To estimate the amount of genetic variation within a population, the most popular variables are allelic richness, the proportion of polymorphic loci, observed heterozygosity and genetic diversity *sensu stricto* (expected heterozygosity) (Schwartz et al. 2007). Departure from

panmixia is usually estimated by calculating Wright's inbreeding coefficient (F_{IS}). When F_{IS} is positive, it can indicate inbreeding from self-fertilization or assortative mating, but it can also result from the spatial structure of the population related to restricted seed or pollen dispersal (Van Rossum & Triest 2006; Fant et al. 2013). Heterozygosity can also be calculated at the individual level if many codominant markers are available (e.g. SNPs), potentially revealing inbred vs outbred individuals (Coulon 2010). The statistical analyses include comparisons between generations, between repeated measures through time, and between translocated and natural populations (e.g. Schwartz et al. 2007; Zavodna et al. 2015; Van Rossum et al. 2020). General(ized) linear models and multivariate analyses can also integrate demographic and environmental data (Rellstab et al. 2015).

To describe genetic divergence among generations and among translocated/natural populations, genetic distance and differentiation statistics (e.g. F_{ST}, G_{ST}, Jost's D) are usually combined with Bayesian clustering analyses and/or multivariate analyses (Appendix S1; Jombart et al. 2010; Alonso et al. 2014; Fotinos et al. 2015; Rodríguez-Rodríguez et al. 2018; Van Rossum et al. 2020). To distinguish between temporal (life stages, generations) or spatial (populations or subpopulations) components of the genetic differentiation, a hierarchical AMOVA can also be performed (Ramp et al. 2006; Van Rossum & Triest 2006; Van Geert et al. 2008).

3. Contemporary gene flow, a key factor for population sustainability

Contemporary gene flow among populations and gene dispersal within populations are key factors for long-term population sustainability (Weeks et al. 2011; Ottewell et al. 2016). The translocation in the field must be designed to optimize random mating and cross-pollination,

by transplanting a large number of individuals, with a high transplant density, and spatially randomizing the sources (Kirchner et al. 2006; Colas et al. 2008; Zavodna et al. 2015; Maschinski & Albrecht 2017). The patterns of gene dispersal in the new recruits is expected to reflect this design. However, different factors may influence gene dispersal, such as life history traits (e.g. seed and pollen dispersal abilities), pollinator service, management interventions, and population demographic dynamics, in terms of survival, flowering and recruitment (Hardy et al. 2004; Raabová et al. 2015; Van Rossum et al. 2015; Benthien et al. 2016). Restricted seed or pollen dispersal may lead to spatial genetic substructuring of populations, with individuals at close geographic proximity being more genetically related, and to local (biparental) inbreeding (Vekemans & Hardy 2004; Van Rossum & Triest 2006).

3.A. Estimation of gene dispersal in translocated populations in practice

Contemporary gene dispersal patterns have only been investigated for a few translocated plant populations (Table 2). Non-random gene flow resulting in spatial genetic structure has been reported 30 years after translocation for *Cochlearia polonica* (Cieślak et al. 2007), and for *Cirsium pitcheri* (Fant et al. 2013). Despite effective pollen dispersal in translocated populations of *Arnica montana*, some spatial structuring appeared in the recruits due to restricted seed dispersal, allowing (half)siblings to grow at close proximity (Van Rossum et al. 2020). This emphasizes the importance of maintaining extensive pollen flow, and so pollinator service for animal-pollinated species, but also of possibly increasing seed dispersal, e.g. by implementing grazing during the fruiting season (Benthien et al. 2016).

3.B. Direct and indirect methods to measure contemporary gene flow

A direct quantification of gene flow can be accomplished by genotyping offspring and their maternal plants to calculate outcrossing, biparental inbreeding and selfing rates (Ritland

2002; McClure & Whitlock 2012). When plants are mapped and sampled exhaustively, paternity analyses can be performed on seeds directly collected on plants to identify pollen donors and distance of pollen dispersal, and possible pollen migrants (Chybicki 2018). When the maternal plant is unknown (in case of recruits), parentage analyses will infer both pollen and seed dispersal. In the case of a large number of potential fathers or parents, high polymorphism of molecular markers is necessary for successful paternity/parentage, and so the analyses can be expensive (Hardy et al. 2004; Chybicki 2018; Flanagan & Jones 2019). Bayesian clustering analyses or Bayesian assignment (Appendix S1) can also identify migrants when the geographic location of the individuals is known and the source populations are genotyped and well differentiated (Aavik et al. 2013).

Indirect methods, which require fewer molecular markers and non-exhaustive sampling, also allow for estimating gene dispersal: the most popular approach is the analysis of spatial genetic structure (SGS) at a fine geographic scale (Hardy & Vekemans 2002; Vekemans & Hardy 2004). The SGS analysis can give insight into gene dispersal patterns for the newly produced generations. The translocated generation is expected to be characterized by an absence of fine-scale SGS when the translocation protocol has led to randomization of the spatial distribution of seed sources. However, depending on plant mortality and on local selective processes, fine-scale SGS in the longer term might appear in the transplants (Table 2; Van Rossum et al. 2020). Separate spatial autocorrelation analyses can be performed within and between different generations (Van Rossum et al. 2020). Indirect estimates of contemporary pollen dispersal can also be obtained from the pattern of pollen pool differentiation between maternal families (Robledo-Arnuncio et al. 2007).

When successful pollination depends on animal vectors, whether pollinator guilds might represent a limiting factor for gene flow can be evaluated by observations of abundance and movements of the visiting pollinators (Pasquet et al. 2008; Brunet et al. 2019). Fluorescent powdered dye particles can be also used as analogues for pollen and provide an estimate of pollinator movements and pollen dispersal distances (Van Rossum et al. 2011, 2015; Diniz et al. 2019). These low-cost methods can be quite convenient when populations are too large for paternity analyses.

4. Mode of local recruitment (sexual or clonal)

The establishment of new plants in the translocated populations can result from sexual reproduction. For many perennial plant species, it can also result from clonal (asexual) propagation. Sexual reproduction can facilitate gene exchanges, and bring new genotypes that will increase mating opportunities and effective population size. It is therefore a good indicator of successful population restoration. Clonal propagation can rapidly provide new individual clones, allowing the maintenance of already existing genotypes and increasing census population size (Menges 2008; Becheler et al. 2017; Van Rossum & Raspé 2018). However, the clumping of clones of a same genotype can also increase self-pollination, and the risk of inbreeding depression. Sexual versus asexual prevalence in recruitment has only been tested for translocated populations of the clonally-propagating *Arnica montana*. The recruitment was mainly based on sexual reproduction, indicating successful population rejuvenation (Van Rossum et al. 2020).

Whether the recruits resulted from sexual reproduction or clonal propagation can be assessed by identifying the distinct multilocus genotypes and assigning each individual to these

genotypes using a probability estimator (Peakall & Smouse 2006). When only a subsample of the transplants is genotyped, the rate of clonality can be quantified using a Bayesian approach that compares transplants with the first new generation (Becheler et al. 2017).

5. Population integration through admixture

Genetic variability and fitness can be maximized by recombining genomes, thus by admixture between transplants (Maschinski et al. 2013; Zavodna et al. 2015; St Clair et al. 2020). Failure in admixture might indicate outbreeding depression or reproductive isolation (Edmands 2007). Therefore, when using nonlocal or several sources for reinforcement and (re)introductions, it is important to verify whether the genetic mixing is effective (i.e. successful mating between local-nonlocal and/or between mixed sources), but also whether the local gene pool has been conserved.

5.A. Assessing admixture in practice

The 12 year-old translocated populations of *Arenaria grandiflora* showed 80% of genotypes resulting from admixed (nonlocal-local) crosses. The local genetic pool was preserved (Zavodna et al. 2015). In translocated populations of *Arnica montana*, 25-68% of the F1 generation resulted from admixture between differentiated seed sources, indicating extensive pollen flow (Van Rossum et al. 2020). In *Castilleja levisecta*, there was indication of mixed parentage between seed sources in reintroduced populations (St. Clair et al. 2020).

5.B. Guidelines for estimating admixture in the newly produced generations

The (admixed) origin of newly established individuals can be estimated by performing Bayesian clustering analyses (Pritchard et al. 2000; St. Clair et al. 2020), which can be followed by a simulation allowing the assignment of the offspring genotypes to one of the

sources or to the first generation hybrids (Van Rossum et al. 2020); by calculating a hybrid index for each individual (Zavodna et al. 2015); by performing a principal coordinate analysis (using the axis values as estimators), and/or by parentage analyses (Peakall & Smouse 2006; Flanagan & Jones 2019).

6. Increase of local plant fitness by genetic rescue and indication of inbreeding or outbreeding depression and local adaptation

When plant translocation goal is genetic rescue, we expect an increase of plant fitness in reinforced -formerly inbred- populations due to the introduction of new genetic variation and/or heterosis (increased vigor of the cross progeny) (Bell et al. 2019). For (re)introduced populations, plant fitness should be higher or similar to reference populations (Weeks et al. 2011). It is also important to check for inbreeding and outbreeding depression (reduced fitness in inbred or admixed offspring), and for local adaptation (higher fitness of local genotypes) (Edmands 2007; Menges 2008). Up to now these evaluations have been based on phenotypic traits but the new genomic technological developments (section 6C) certainly offer future perspectives in detecting heterosis, inbreeding / outbreeding depression or selection processes.

6.A. Evaluation of genetic rescue, inbreeding or outbreeding depression and local adaptation in practice

Genetic rescue as well as inbreeding and outbreeding depression may be tested by experimental crosses (e.g. Edmands 2007; Willi et al. 2007; Bartmentlo et al. 2018), and local adaptation by reciprocal transplant experiments (Bowman et al. 2008; Reckinger et al. 2010). However, such experiments are not always possible to carry out before implementing plant translocations in species recovery plans, as it may take several years to obtain new

generations. Genetic rescue, using local or nonlocal source provenance is usually successful (Table 2; Wili et al. 2007; Betz et al. 2013; Zavodna et al. 2015; Bartmentlo et al. 2018). However, the effects of heterosis, inbreeding and outbreeding depression and local adaptation on plant fitness may be complex and conflicting, and may depend on life history stages (Bowles et al. 2015; Zavodna et al. 2015; Bartmentlo et al. 2018). There was no sign of heterosis or outbreeding depression in the F1 generation of translocated populations of *Arnica montana*. Phenotypic plasticity and maternal effects were found (Van Rossum et al. 2020). Phenotypic plasticity may increase transplant survival in spatially or temporally varying environments in the short term, and favor adaptation and population evolutionary resilience to changing environmental conditions in the long term (Nicotra et al. 2015; Christmas et al. 2016). Maternal effects may favor adaptation in stable environments (Schuler & Orrock 2012). Differences in progeny fitness may also reflect genetic variability (Basey et al. 2015; Hamilton et al. 2017), and so contribute to population evolutionary potential.

6.B. Guidelines for evaluating genetic rescue, inbreeding or outbreeding depression and local adaptation using phenotypic traits

Including molecular data in the analyses of individual phenotypic traits (Table 1) can make the inferences stronger (Zavodna et al. 2015; Bartmentlo et al. 2018). To avoid variation related to environmental effects and examine possible maternal effects, measurements should be preferably carried out in standardized environments (Leinonen et al. 2008; Whitlock 2008), but that remain as close as possible to the environments of natural or translocated populations. It is also possible to measure traits in the field provided a large number of individuals has been translocated to a site, and that the population origin of the transplants is known (Savolainen et al. 2013). Defining the environmental conditions in controlled

experiments may be important as inbreeding depression or heterosis effects are not always expressed when the conditions are optimal (e.g. in case of cultivation in common garden), but appear under stressful conditions, such as dryness or competition (Edmands 2007; Willi et al. 2007). Phenotypic plasticity may also not be expressed in a common-garden environment (Whitlock 2008). The phenotypic data can be analyzed in relation to their molecular genotypes (local - introduced genotypes, individual heterozygosity, admixture level), source origin, and environmental characteristics of the sites, including management interventions, but can also be compared with data from natural (source, inbred and healthy) populations (Bowman et al. 2008; Reckinger et al. 2010; Bowles et al. 2015; Zavodna et al. 2015).

When inbred and outbred (admixed or not) individuals can be clearly identified based on their molecular genotypes, inbreeding or outbreeding depression can be quantified by calculating a relative performance coefficient (Angeloni et al. 2011). Local adaptation can be quantified by calculating the relative fitness of translocated individuals compared with natural ones or between different sources at a given translocation site and in a given year (Hereford 2009).

6.C. Guidelines for evaluating genetic rescue, inbreeding or outbreeding depression and local adaptation using genomic markers

To detect evidence of local adaptation, different approaches may be used. First, a genome-wide selection scan analysis compares genetic variation among all genomic markers to build a neutral distribution. The markers deviating from this distribution are expected to be under selection (linked to candidate adaptive genes) (Angeloni et al. 2012; Benestan et al. 2016). Second, genotype-environment association methods consist of identifying candidate adaptive loci through association between allele frequencies and environmental variables, by

performing univariate, multivariate or Bayesian analyses, possibly combined with geographic information systems (Joost et al. 2007; Flanagan et al. 2018). Finally, genome-wide association studies test the association of genotypes with phenotypic data (Savolainen et al. 2013; Rellstab et al. 2015). The homozygosity/heterozygosity level of some genomic regions may also be associated with fitness traits, revealing major genes implicated in inbreeding/outbreeding depression (Angeloni et al. 2012).

7. Long-term monitoring of genetic changes

A short-term monitoring over a few generations can give an indication of contemporary processes, such as pollination and seed dispersal on yearly basis. However, long-term genetic monitoring is also necessary, because the genetic composition of a translocated population can change through time. First, outbreeding depression can only be expressed in the progeny after two or three generations of admixture (Edmands 2007). Second, it can take time for natural selection to remove the poorly adapted genotypes (Week et al. 2011; Zavodna et al. 2015; Barmentlo et al. 2018). Finally, the temporal dynamics of the genetic composition can depend on species life history traits (e.g. breeding system, growth form, plant longevity, recruitment time lag), translocated population demographic dynamics (e.g. census population size, plant density, sex or morph ratio, recruitment rate) and variation in local factors between translocated sites (e.g. management interventions, soil chemical composition, vegetation composition, grazing pressure, competition) (Van Rossum & Triest 2006; Bowman et al. 2008; Menges 2008; Weeks et al. 2011; Maschinski & Albrecht 2017; Albrecht et al. 2019). Therefore, measurements (possibly restricted to those identified as key indicators in the restoration of genetically viable populations) should be repeated over several generations and

over a long period of time, for detecting possible changes in genetic diversity and structure, rescue status and adaptive response.

Despite their importance in evaluating the success of plant translocations, long-term monitoring studies over 10-20 years are still rare for demographic surveys (Colas et al. 2008; Godefroid et al. 2011; Albrecht et al. 2019), and even scarcer for genetic monitoring (Bowles et al. 2015; Zavodna et al. 2015; Rodríguez-Rodríguez et al. 2018). A long-term genetic survey may be difficult to implement for understandable reasons (limited budget and staff or other conservation priorities for practitioners). Given that plant translocations may be the last chance for preserving some populations and represent high financial and time investment (Fenu et al. 2019), it should be systematically considered in species recovery plans.

Repeated, long-term molecular data collection allows the calculation of two indicators of the long-term sustainability of the populations: effective population size (N_e) and population viability. To our knowledge, one attempt has been made to estimate these indicators on translocated plant populations, for the clonal *Grevillea scapigera* (Table 2). Despite demographic data indicating translocation success with thousands of *G. scapigera* seeds produced by a large census population size (266 plants), N_e estimates were approximately 2 because only 10 plants were used as a source and there was large variation in reproductive success (Krauss et al. 2002). N_e can be estimated using the linkage disequilibrium estimator (comparison of N_e over several years), temporal changes in allele frequencies, and sibship/parentage frequency (Luikart et al. 2010; Wang 2016; Appendix S1). A Population viability analysis (PVA) is a model to estimate the size needed for the persistence of populations over time and their risk of extinction (Menges 2008; Pe'er et al. 2013). When

combining demographic, genetic and environmental data through time and space, the PVA can take the evolutionary potential into account (Kirchner et al. 2006; Pierson et al. 2015).

A tool for conservation practitioners

Genetic monitoring is a useful tool for evaluating whether species conservation plans have achieved the recovery of long-term sustainable translocated populations. However, the available genetic tools are often underused. Attention should be given to recruitment by sexual reproduction, contemporary gene flow, admixture between sources and the maintenance of the local genetic pool. These factors are also important to consider if the translocated populations will be used as sources for further translocations. We believe best practices for monitoring conservation translocations should include both molecular and phenotypic approaches, given the potential roles of heterosis, outbreeding and adaptation but also of phenotypic plasticity and maternal effects on population dynamics. Results of genetic monitoring studies (Betz et al. 2013; Van Rossum et al. 2020) emphasize the importance of implementing ecological management interventions to stimulate flowering that optimize pollination and random mating, and thus reproductive success, and to favor seed germination and recruitment, so that genetic dynamics can be initiated and further pursued with success and maximize evolutionary resilience. The guidelines in the present paper will help conservation practitioners to find the appropriate genetic survey methods depending on the goal of the plant translocation, so that they can adapt management practices to better integrate evolutionary processes.

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Supporting Information

A non exhaustive list of software that can be used for molecular analyses to implement genetic monitoring of translocated plant populations (Appendix S1), and literature cited in Table 2 (Appendix S2) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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Table 1. Quantitative traits to measure plant fitness at individual level (from Willi et al. 2007; Menges 2008; Angeloni et al. 2011; Bowles et al. 2015; Zavodna et al. 2015; Barmentlo et al. 2018; Godefroid & Van Rossum 2018).

Fitness trait	Variable to measure
Health	Seedling chlorosis
	Plant mortality
	Disease
	Herbivory
Vegetative performance	Plant size and height
	Growth (including clonal production)
	Number of leaves
Reproductive	Floral display

performance	
	Pollen viability
	Seed set
	Seed weight
	Seed abortion
	Seed germination
Phenotypic plasticity	Variance in the measured characters
Cumulative fitness performance	Seed yield (fruit set x viable seed set x mean seed weight)
	Sexual fitness (viable seed set x germination rate x survival x number of flowers, flowers buds and fruits)
	Leaf area index (number of leaves x leaf length x leaf width)

Table 2. Studies involving genetic monitoring of translocated populations of critically endangered plant species from the literature [a].

Study species	N (n); markers-traits [b]	Main results of the genetic monitoring	Reference [c]
Arenaria grandiflora	3 (440 plants; based on 9 local and 11 non-local cuttings); 10 microsatellites, flower number/plant	Higher genetic diversity in reintroduced populations (mixed sources) than in natural populations, maintained over 12 years; 80% of admixed individuals and local genetic pool maintained after 12 years; conflicting effects of heterosis, inbreeding and outbreeding depressions and local adaptation on plant fitness	Zavodna et al. 2015
Argyroxiphium sandwicense ssp. *sandwicense*	1 (450 plants; seeds from 2 maternal plants); 11 RAPD	Lower genetic diversity in the reintroduced than in the natural population due to the small number of plants (2) used as seed sources; manual pollen transfer from the natural to the reintroduced population recommended to incorporate additional genotypes, followed by natural recruitment	Robichaux et al. 1997
Arnica montana	3 (700 plants; from 2 seed sources); 9 microsatellites, seed fitness performance, plant size	High levels of genetic variation, random contemporary gene flow (but SGS in the recruits as a result of restricted seed dispersal) and admixture (25-68 %) in the first generation of seed progeny and newly established	Van Rossum et al. 2020

		recruits	
Asclepias meadii	7 (30-180 plants and 35-135 seeds; 20-71 plants and 18-66 seedlings after 1 year); leaf area index	After 10 years: heterosis at seedling establishment and outbreeding depression expressed at older stages and under more stressful conditions, but multiple seed sources needed for maximizing self-incompatible allele diversity	Bowles et al. 2015
Banksia attenuata	1 (seeds; local provenance; 200 trees after 13 years); 7 microsatellites, germination, survival, leaf and root traits	Similar high genetic diversity and offspring fitness performance, and low differentiation and inbreeding in restored and natural populations; low SGS and extensive pollen dispersal within and between populations	Ritchie & Krauss 2012
Castilleja levisecta	11 (seeds or plants; from 4 seed sources); 7 microsatellites	Higher genetic diversity and lower inbreeding and relatedness in reintroduced (mixed sources) than in source populations; indication of admixture; better to directly use seeds from sources than producing them by nursery beds	St. Clair et al. 2020
Cirsium pitcheri	2 (100-500 plants 8-15 years after the last translocation; 6 seed sources); 6 microsatellites	Higher genetic diversity in reintroduced (mixed sources) than in natural populations, but higher F_{IS} values as a result of inbreeding and spatial substructuring (and so limited contemporary gene flow); small flowering -and effective- population sizes	Fant et al. 2013
Cochlearia polonica	1 (14 plants; 30,000 after 30 years); 3 AFLPs	Fine-scale spatial genetic structure within the population due to nonrandom gene flow; seed sampling to create new populations must be done on the whole population.	Cieślak et al. 2007
Grevillea scapigera	2 (hundreds of clones from 10 plants; 266 plants survived); 3 AFLPs	Low genetic diversity and high inbreeding in the F1 generation (seed progeny), and N_e = ~2 due to the small number of genotypes used as source for translocation and variance in reproductive success; to increase N_e : use offspring of founders and stimulate germination through disturbance	Krauss et al. 2002
Lasthenia conjugens	192 (3x100 seeds); 3 ISSRs	Similar genetic diversity across 3 generations and among restored and	Ramp et al. 2006

		natural populations	
Lychnis flos-cuculi	9 (commercial seed mixtures; 12-2050 plants after 3 or 8 years); 6 microsatellites	Similar genetic diversity and allelic richness but inbreeding coefficients 3 times higher in sown than in natural populations; restricted contemporary gene flow among sown populations, depending on distance and population size	Aavik et al. 2012, 2013
Prostanthera eurybioides	2 (reinforced with local seeds)	Similar genetic diversity between planted and natural subpopulations	Ottewell et al. 2016
Pseudophenix sargentii	3 (3-119 plants; 0-63 plants after 20 years); 10 microsatellites	Increased genetic diversity in 1 population but evidence of inbreeding in the other one, which needs additional reintroduction	Fotinos et al. 2015
Pulsatilla vulgaris	9 (1-380 plants after 3 years); 3 AFLPs, vegetative and reproductive performance	Self-reinforcement after 3 years has led to similar levels of genetic variation, fitness performance and germination rates for natural and translocated individuals; population sizes have increased (1 to 68-fold)	Betz et al. 2013
Sambucus palmensis	13 (cuttings; sampling 3-129 plants); 30 years reintroduction program; 7 microsatellites	Increased genetic diversity and high clonality due to the propagation method (by cuttings), but indication of natural regeneration by sexual reproduction; still rare alleles in natural populations not present in the translocated ones	Rodríguez-Rodríguez et al. 2018
Silene hifacensis	3 (> 10 years: 4-14 plants); 3 AFLPs	Similar genetic diversity among restored and natural populations; inbreeding in two restored populations likely due to ex situ cultivation history, suggesting to further use mixed seed origins	Alonso et al. 2014

[a] based on an exhaustive review in Web of Science, Google Scholar and Google using plant, conservation, genetic, population, restoration, rescue, translocation, reinforcement, augmentation, (re)introduction, and/or genetic monitoring as keywords.

[b] Abbreviations: N, number of translocated populations, n, number of translocated individuals per population; markers-traits: molecular markers and fitness traits investigated in the study.

[c] For the cited literature, see Appendix S2.

Figure legend

Figure 1. Guideline summary for implementing genetic monitoring to evaluate the success of plant translocations, according to population status and translocation goal [reinforcement (❀) of depauperate or inbred populations or (re)introduction (✿) when populations have gone extinct], and to the sources used for producing transplants (local, nonlocal, mixed).