CSIRO PUBLISHING
www.publish.csiro.au/journals/ajb Australian Journal of Botany, 2007, 55, 362–370

Reintroduction of rare and endangered plants: common factors, questions and approaches

Edward O. Guerrant Jr[A,C] *and Thomas N. Kaye*[B]

[A]Berry Botanic Garden, 11505 SW Summerville Avenue, Portland, Oregon 97219, USA.
[B]Institute for Applied Ecology, 563 SW Jefferson Avenue, Corvallis, Oregon 97333, *and* Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon 97331, USA.
[C]Corresponding author. Email: ed.guerrant@berrybot.org

Abstract. The science of reintroduction for conservation purposes is young, and there is still much to learn about the practice. As a means to achieving biological goals of successfully establishing new populations to enhance a species survival prospects, and project goals, such as learning how to go about establishing new populations, reintroduction projects are best done as well designed scientific experiments that test explicit hypotheses. Focusing on a range of factors common to any reintroduction, we review several empirical reintroduction projects with respect to hypotheses tested, experimental materials and methods employed, and evaluate their success in both biological and project terms.

Introduction

The central biological goal of rare-plant reintroduction is to establish resilient, self-sustaining populations that have sufficient genetic resources to undergo adaptive evolutionary change (Guerrant 1996*a*). In other words, the purpose is to enhance the species survival prospects in the wild. Reintroduction is used here as a general term that includes the establishment of new populations and re-establishment of extirpated populations from *ex situ* material, and the enhancement or augmentation of existing populations. It does not include the translocation (by removal and transplantation) of naturally occurring plants from one location to another, which involves a different set of strategic, procedural and ethical considerations.

In an insightful discussion of how to measure and define success in rare-plant reintroduction, Pavlik (1996) distinguished biological purposes and project purposes. Biological purposes revolve around the desire to establish new or augment existing populations and thus increase a species survival prospects. Project purposes have to do with evaluating the means by which the desired biological ends are pursued. Strategically, and as a practical matter, we agree with Falk *et al*. (1996) that the most efficient way to achieve the biological and project purposes is to conduct reintroduction projects as scientific experiments, carefully crafted to test explicit hypotheses about how best to go about the practice of reintroduction. In that way, whether or not the project is biologically successful, methods and protocols are most likely to be improved by information gained from the effort.

Reintroduction projects typically have multiple purposes. In addition to the basic biological purpose of establishing new or increasing the size or diversity of existing populations, project goals may include evaluations of practical greenhouse or field methods to theory-driven hypotheses about demography, population genetics or ecological interactions. By designing reintroduction projects as controlled scientific experiments, the effects of particular factors can be elucidated. Through careful observation and monitoring, additional, supplementary information can often be gleaned opportunistically. Reintroduction projects can also serve public education and policy purposes, both to provide the public with a focal point for what otherwise can be abstract discussions about the plight of rare species, and to give policy makers information with which to make better, more informed choices.

Different legitimate purposes can conflict with one another (Guerrant *et al*. 2004). What may seem important to address as a matter of scientific interest may not be in the best conservation interest of the species in that place and time. For example, the results of a common-garden experiment, in which material collected across a species range are compared, might be interesting theoretically and have significant practical implications, but could degrade the survival prospects of the resulting population through outbreeding depression or introduction of genes maladapted to local conditions. It is important, therefore, not only to be clear about the various purposes served by any reintroduction attempt, but also about their relative priority, and to anticipate potential unintended consequences, such that critical values are not compromised.

Each reintroduction project is unique with regard to the species involved, questions asked, intended purposes and external circumstances in which the work is conducted. Nevertheless, a large number of basic and important factors or elements are common to many if not all reintroduction attempts, and are thus incorporated into projects, either explicitly or implicitly.

The central focus of this paper is on a set of elements common to many if not all reintroduction projects, and how we have addressed them as research questions into our work. Table 1 lists some of these, and how they were incorporated into seven

 10.1071/BT06033 0067-1924/07/030362

Table 1. Factors common to many reintroduction attempts, and how or whether they were part of projects with particular taxa
Entry meanings for several factors explained in table itself. The letter 'Y' indicates yes, that factor was an experimental variable in that study, and 'N' indicates no, that factor was not an experimental variable

Parameter	*Abronia*	*Arabis*	*Castilleja*	*Erigeron*	*Horkelia*	*Lilium*	*Lomatium*	*Lupinus*	*Sericocarpus Aster*	*Stephanomeria*
Propagule type: seeds (S), transplants (T)	S/T	S/T	T	S/T	S/T	S/T	S/T	S/T	S/T	T
Source material: one or more populations (seed, transplant)	1,1	1,1	6	1,3	1,2	4,4	1,1	1,1	1,2	1
Material wild collected (W), or from stock propagated off site (P)	W	W	W	W	W	W	W	W	W	P
Number and relatedness of founders. Maternal lines maintained separately (ML) or bulk (B) collection from multiple plants?	B	ML	ML	B	B	ML	B	B	B	B
Test fit of different source populations at site	N	N	Y	Y	Y	Y	N	N	Y	N
Reintroduction site: geographical location in absolute terms, and in relation to extant or extirpated populations	N	N	Y	N	N	N	N	N	N	N
Habitat observational data: slope, aspect, vegetation, soils, etc.	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Habitat site manipulation										
Pre-planting site preparation (*cleared ground of vegetation at time of planting, in seed trials only)	Y	N	Y	Y*	Y*	Y	Y*	Y*	Y*	Y
Soil Amendments	Y	N	N	Y	Y	N	Y	Y	Y	N
Post planting care: water (H), weeding (W), pest control (PC), vegetation management (VM), or none (N)	N	N	PC	N	N	VM	N	N	N	W
Timing										
Season	W	F	F/S	F/S	F/S	F	F/S	F/S	F/S	S
Number of attempts	>5	1	2	2	2	1	2	2	2	2

separate projects involving 10 species, which one or the other of us has conducted. All projects have been in the State of Oregon, which is located between 42° and 46°N latitude in the Pacific north-west region of the United States (Fig. 1). Not all of these factors will necessarily be a variable in any given project. Even so, whether or not a particular factor varies, or is even explicitly considered in any particular project, decisions about these and other components must be made. We preface the discussion of various factors involved in reintroduction, with brief outlines of the various projects and species with which we have worked. Guerrant was involved in three projects, each of which focused on a single species, namely *Arabis koehleri* T.J.Howell var. *koehleri* (Brassicaceae) (Guerrant 2005*a*), *Lilium occidentale* Purdy (Liliaceae) (Guerrant 2001, 2005*b*) and *Stephanomeria malheurensis* Gottlieb (Asteraceae) (Guerrant 1996*b*; Guerrant and Pavlik 1997; Parenti and Guerrant 1990). Kaye was involved with three other projects involving seven species. Projects with single species were conducted with *Abronia umbellata* Lam. ssp. *breviflora* (Standl.) Munz (Nyctaginaceae) (Kaye 2003, 2004), *Castilleja levisecta* Greenm. (Scrophulariaceae) (Kaye and Lawrence 2003; Lawrence 2005; Lawrence and Kaye 2006) and *Lupinus sulphureus* Douglas ssp. *kincaidii* (C.P. Smith) Phillips (Fabaceae) (Kaye and Cramer 2003; Kaye and Brandt 2005). Four species, namely *Erigeron decumbens* Nutt. var. *decumbens* (Asteraceae), *Horkelia congesta* Dougl. ex Hook. ssp. *congesta* (Rosaceae), *Lomatium bradshawii* (Rose ex Mathais) Mathias & Constance (Apiaceae) and *Sericocarpus rigidus* Lindl. (syn. *Aster curtus* Cronquist., Asteraceae), all occurred in the same wet-prairie habitat, and are part of a larger project (Kaye and Brandt 2005).

These species and projects represent a variety of plant families and life forms, and the projects were conducted across a diversity of very different habitat types and climatic regimes (Fig. 1). The habitats in which these species grow range from exposed, sandy ocean beaches to low-lying openings in coastal temperate forests with seasonally saturated soils, wet prairies, well drained rocky outcrops in interior mountains and arid high desert sage brush steppe. Prevailing westerly winds bring moisture and moderating temperatures off the Pacific Ocean, resulting in a Mediterranean-type climate in Oregon. The three climatograms in Fig. 1 show how the bulk of precipitation falls in the cooler months, and temperatures become more extreme with increasing distance from the coast. Note also a significant rain-shadow effect east of the Cascade mountain range. Materials and methods used in specific studies will be outlined where needed to explain or put the results in context.

Elements common to many reintroduction projects

Myriad factors affect the success or failure of a reintroduction attempt, and not all can be anticipated, much less taken into consideration or be subject to controlled experimentation. Nevertheless, many biological and methodological factors are common to most if not all reintroduction attempts (Table 1) and amenable to experimental examination. Biological factors include propagule type, characteristics of the reintroduction site, and the number and location of source populations of founding stock. Methodological factors include techniques for handling and planting propagules, site preparation, post-planting care, addition of soil amendments, and so on. In any given project, not all of these factors will be variables to manipulate or examine, but decisions about them and other factors must often be made.

The remainder of this article will be devoted to discussion of common factors in reintroduction projects by using data from our own studies.

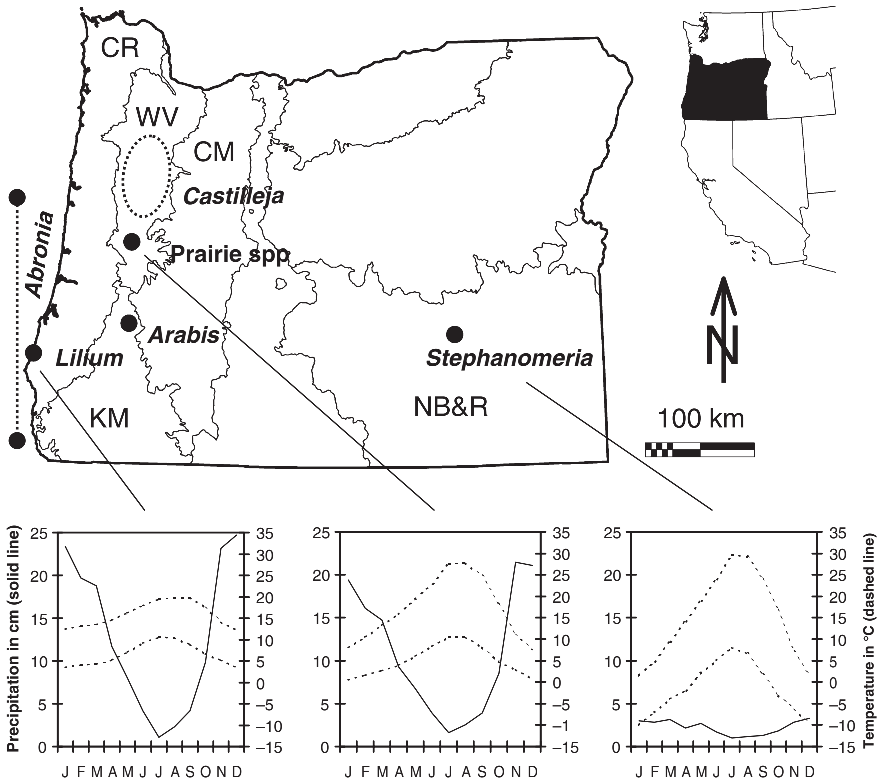

Fig. 1. Base map of the State of Oregon, with boundaries indicated for US Environmental Protection Agency ecoregions, level III (http://www.epa.gov/wed/pages/ecoregions/level_iii.htm). Ecoregions in which reintroductions were conducted are as follows: CR, Coast Range; WV, Willamette Valley, KM, Klamath Mountains; and, NB&R, Northern Basin and Range. Reintroduction sites are indicated with a closed circle (Prairie species locality includes *Lupinus* site), the range of coast of the various *Abronia* sites are indicated by a straight dotted line, and the area in which the *Castilleja* reintroduction sites were located is indicated by a dotted line oval. To the east of the WV and KR is a major mountain range, the Cascade Mountains (CM). Climatograms indicating monthly mean precipitation and high and low temperatures are provided for long-term (1971–2000) weather stations in the vicinity of three of the reintroduction sites (data for Bandon, Eugene, and Burns, Oregon on the website of the Oregon Climate Service. http://www.ocs.oregonstate.edu/index.html).

Propagule type: seeds or transplants

Perhaps the most basic decision is which propagule type to use: seeds or transplants, a category that can include plants with very different pre-transplanting histories. We use the term transplants to mean outplanted greenhouse-grown starts. All but two of the reintroduction studies with which we have been involved used both seeds and transplants as founders (Table 1). In general, transplants yielded greater establishment rates than did the seeds (Table 2), but in some cases seeding was an effective and cost-efficient method of establishing substantial numbers of plants. Simple differences in relative establishment of seeds and transplants do not tell the whole story. When choosing which propagule type to use, other factors must be considered, including propagule availability, age (time in storage) and relative financial and other resource costs associated with using seeds *v.* transplants.

After 9 years of an ongoing reintroduction project with *Lilium*, an iteroparous herbaceous geocarpic plant, plants established as bulbs consistently emerged in significantly greater proportion than those from both new and old seed (Guerrant 2001, 2005*b*). Except for the first year, plants from new seeds consistently emerged in slightly higher proportion than did plants from old seeds (Table 2), and in some but not all years, the differences were significant. New seeds (NS) were planted the year they were formed, and old seeds (OS) were seeds that had been stored for 1 or 2 years in the Berry Botanic Garden's seed bank. Plants from old seeds, however, grew larger than those from new seeds, so neither new nor old seeds appeared to be the clearly superior option. These differences suggest that

Table 2. Summary results for nine species using both seeds and transplants, comparing establishment and survival rates (over what period of time)

Numbers in seed and transplant columns indicate percent survival

Species	Seed (%)	Transplants (%)
Abronia	0.5	16–76
Arabis (3 years)	0.0	10
Erigeron (5 years)	0.2–0.5	19–20
Horkelia (5 years)	2–4	32–38
Lilium (1, 5 and 9 years)	NS 23, 42, 26	90, 61, 43
	OS 48, 34, 23	
Lomatium	20–25	27–54
Lupinus (1 year)	24	88
(Isabel, Coble) (5 years)	Scarified 4	0–36
	Not scarified 5–6	
Sericocarpus (5 year)	1–2	23–81

something may have happened during storage that affected both germination (first positively, and then over time and overall, negatively) and growth rates (positively), a topic that deserves more attention in this and other species. Alternatively, it may simply reflect intrinsic differences in the seed lots among years, but this seems unlikely as the old seed was from collections in two different years at the same site, and they behaved more similarly to one another than either did to the new seed. Among other things, this study shows that the effects of different propagule types, and their histories before planting, can be complex, subtle and take many years to become fully expressed.

In another study, of the iteroparous woody shrub, *Arabis koehleri* var. *koehleri*, 567 seeds and 189 transplants, all from the same nine maternal lines, were individually planted, mapped and marked in the field in the fall of 2001 (Guerrant 2005*a*). Presumably because the summers of 2002 and 2003 were much drier than average, only ~10% of the transplants survived the first 2 years after planting, but there was no additional mortality in 2004 or 2005. Although some seed germinated, no plants derived from seed survived the first summer in the field (Guerrant 2005*a*). Although transplanting was a superior technique in this case, unpredictable environmental conditions can have profound effects on reintroduction success. Qualitatively similar results were found in a set of experiments involving four rare or endangered Willamette Valley prairie species (*Erigeron*, *Horkelia*, *Lomatium*, *Sericocarpus*), all iteroparous herbaceous perennials (Kaye and Brandt 2005). Five years after sowing, plant establishment from seed ranged from <1 to 25%, whereas transplant survival spanned from 19 to 81%, depending on the species and treatment (Table 2). Establishment from seed was relatively high in *Lomatium* in particular (25% averaged across weeding treatments) and transplant success was only slightly higher (27% averaged across treatments) after 4 years. In contrast, establishment of *Erigeron* from seed was less than 1% but transplant success exceeded 20% at the end of the study. Even among species with relatively similar life histories that had grown in the same habitat, the relative success of seeds *v.* transplants can differ substantially.

The decision to use seeds or transplants is not simply a function of relative establishment and survival rates. Additional factors must be considered, such as how much seed is available for collection without unduly affecting harvested populations (Menges *et al*. 2004), and also the economic cost of using seeds *v.* transplants as founders.

Abronia umbellata ssp. *breviflora* is an annual to short-lived (2 years) perennial herb of sandy beaches exposed to winter storms. Some individuals are prolific seeders, producing many thousands of one-seeded fruits. It is known from relatively few, small populations, scattered along the Pacific Coast of North America, from northern California, through Oregon and historically into Washington and British Columbia, Canada. Not surprisingly, in experiments at over 15 beaches and across more than 10 years, transplants established at much higher rates than seeds sown directly on beaches (47% *v.* 0.5% on average) (Kaye 2003, 2004). Although direct seeding with small (5000) or large (50 000) numbers of seeds resulted in about the same average establishment rates, reintroduction failure (zero established plants) was more frequent with small seed doses than with large numbers of seeds. Even though orders of magnitude higher establishment rates achieved for transplants relative to seeds, greater costs in time and other resources were incurred when transplants were used. In addition, the great abundance of available seeds makes using seeds a reasonable choice in this species.

The costs of reintroduction using either seeds or transplants can be measured in multiple currencies. In an experiment with the threatened *Lupinus sulphureus* ssp. *kincaidii*, which is the larval host plant of the endangered Fender's blue butterfly (*Icaricia icarioides fenderi*), Kaye and Cramer (2003) evaluated establishment costs both monetarily and in terms of seeds. Direct seeding was much more economical than was transplanting, when measured as leaf production per dollar (14.3 *v.* 0.4) and in inflorescences per dollar (0.9 *v.* 0.1). In terms of seeds used, however, direct seeding was much less economical than was transplanting. Leaf production per seed was much lower from direct seeding than transplanting (7.7 *v.* 41.6), as was inflorescence production (0.5 *v.* 1.0).

The plentiful seed production of *Abronia* makes it seeds a practical choice to be used as founders, despite very low absolute establishment rates. In contrast, transplanting of *Lupinus sulphureus* ssp. *kincaidii* may be a better choice than seeding because wild seeds are often produced in very limited numbers and seeding is less efficient than planting for leaf and flower production. If large numbers of seeds are available (and we recommend a managed seed increase program for this species), direct seeding may be more cost effective.

Source population: single or multiple?

The decision to use seeds from one or more source populations is inherent in every study, either explicitly or implicitly. Context dependent, no single correct choice applies to all situations. In some situations, of course, only one option may be available. The following examples illustrate a variety of situations in which one or more source populations were used (Table 1), and some of the factors that were considered in each case.

Single seed source

The primary reasons behind the use of a single seed source are usually either practical, i.e. only one source is available, or biological—using more than one source could cause some unwanted harm. *Stephanomeria malheurensis* is an herbaceous annual inbreeder so rare that it is known only from a single population in Oregon. It had apparently become extinct in the wild in 1985–1986. *Ex situ* seed was available at the Berry Botanic Garden, which used it for reintroduction back into the wild in 1987 (see Parenti and Guerrant 1990; Guerrant 1996*b*; Guerrant and Pavlik 1997). Its habitat is arid sagebrush-steppe, with highly stochastic rainfall, and the population fluctuated and decreased and went extinct a second time. A second reintroduction attempt is planned, and it will use propagated seed from the original single source.

Some species may remain at multiple sites, but have seed production sufficient to support harvest at only one location. Such is the case with *Abronia*. Reintroductions in Oregon with this species have relied on one source, but with a twist. A population at Port Orford numbered over one thousand individuals in the late 1990s, and seeds were collected there for large-scale reintroductions elsewhere on the coast. Since then, that population declined but one of the reintroduced sites flourished and became the new seed source (Kaye 2004), and genetic evaluation showed that this reintroduced population captured the genetic variability of the original source (McGlaughlin *et al*. 2002). Finally, and in the absence of compelling genetic or demographic reasons for using multiple populations, using a single seed source may be necessary or desirable for experimental reasons. In studies with *Arabis*, for example, only one source was used to keep the number of factors in the experimental design manageable.

Multiple seed sources

Other projects discussed here have for the most part used multiple-source populations (Table 1). There is considerable variation among projects in the number of and reason for multiple sources, as well as their geographical relationship to the reintroduction sites and to other conspecific populations.

Three of the four prairie species, all except *Lomatium*, used multiple-source populations to test the hypothesis that even at a local scale, variation in source performance could affect plant size and survival, and therefore project success. All collections were made from within a roughly 4 by 10 km area that also included the reintroduction sites. Only in *Horkelia* was there any indication of source-population effects on survival, and that was a weak effect in a single year. T. N. Kaye (unpubl. data) has since established another experiment explicitly to examine in *Horkelia* the effects of single-source *v.* mixed-source populations on seed set and long-term seedling recruitment. In a separate experiment with *Castilleja levisecta* (Scrophulariaceae), an iteroparous herbaceous perennial extant in Washington and British Columbia but extinct in Oregon, six source populations were grown together in nine common gardens in Oregon to evaluate which seed sources performed best as reintroduction material (Lawrence 2005; Lawrence and Kaye 2006). This approach identified three sources that could be expected to do well in the extirpated portion of the species range.

In the *Lilium* introduction, four source populations were chosen for their geographical proximity and ecological similarity, mostly with respect to soil type and associated vegetation. The species has a long, ~320-km long, but narrow coastal distribution, having been found no more than 6 km inland, and with a rather patchy pattern of occurrences separated by long distances. Consequently, *Lilium* source populations were somewhat further from the reintroduction site than were those of *Arabis* or the prairie species, at a distance of between 10 and 40 km, and all to the north of the reintroduction site. The reintroduction site is located towards the middle of the north–south range, from which many populations have become extirpated.

The choices of how many and which source populations to use do not occur in isolation. They are made in the context of many other factors, including the intended reintroduction site and its proximity to other populations. Despite the fact that seeds were available, and that it would have been interesting scientifically to use as founders individuals from across the entire range of *Lilium*, we chose to use only the most local and ecologically similar source populations. This decision was due in part to the possibility that other naturally occurring populations might still be found in the general area. Indeed, in the years since reintroduction, two populations have been found in the general area, at least one of which is within a distance that the hummingbird pollinators might move. Source-population effects on emergence were noted for the first 3 years after planting, and not again for another 4 years, until 2003, a year in which the fewest plants were seen. The source population with the least emergence in the first year has since been the one that has emerged in the greatest amounts. Conversely, the population that did best the first year, did much more poorly in 2003 than the other three populations. A significant effect of source population on plant size was not noted until 3 years after planting, and persisted for three more years, after which it was no longer detected.

The contrasting effects of source population on emergence and plant size in *Lilium*, and that the effects change over time, suggest the effects of source population on biological success may often be subtle, complex, and take years to express themselves.

Number and relationship of founders

Like source population number, the number and relatedness of founders used in a reintroduction project are inherently factors. All else being equal, the more founders, the greater will presumably be the chance that some will establish and survive (Falk *et al*. 1996; Guerrant 1996*a*). In this context, relationship among founders refers to whether the founders are of known maternal line (i.e. sibs or half sibs), or from a bulk collection (whether from a known or unknown number of maternal lines.) Both the number of propagules used, and our ability to track their familial relationships vary among projects.

The fates of propagules from different maternal lines were followed separately in the *Lilium* and *Arabis* projects, but not in the others, where bulk collections were used. There is no universally correct choice. There are benefits and costs associated with the use of propagules of known parentage, as there are with the use of propagules from bulk collections (of known or unknown maternal lines).

There are good theoretical reasons having to do with maximising effective population size for a given census size why it is advantageous to maximise the number of founding individuals, and to equalise the contribution of each, and these are backed with empirical results (Loebel *et al*. 1992; Borlase *et al*. 1993; Guerrant 1996*a*; Newman and Pilson 1997). This level of control is possible only if seeds from particular maternal parents are collected and maintained separately before use. Such detailed information on the composition of the founding population also allows for finer-scale studies of the genetic basis for differences in establishment, growth and reproductive success.

The 760 propagules used in the *Lilium* project came from a total of 81 maternal plants, from four populations. The 320 new seeds, planted the year they were produced, came from 31 plants (different maternal lines contributing 7–12 seeds each). The 320 'old seeds' and 120 'bulbs' came from the same set of 50 maternal lines, collected over a period of 2 years, and had been dried and stored in the Berry Botanic Seed Bank for 1 or 2 years. Of the 320 old seeds used, 49 maternal lines supplied between five and eight seeds each. Of the 120 bulbs used, 34 maternal lines supplied between 1 and 14 seeds each (all but 2 of which contributed 7 or fewer), of which only one maternal line did not also supply old seeds for the project. Effects of maternal lines differ among propagule types, and between survivorship and growth rates. Maternal family had no discernable effect on survival prospects of bulbs, but did, more years than not, on both old and new seeds. Conversely, maternal family had a statistically significant effect on leaf area, a measure of plant size and growth rate, on bulbs, and except for new seeds in the first year after planting, maternal family did not affect the size of plants from either old or new seeds.

The four Willamette Valley prairie species used bulk collections from greater than 20 individuals from each population sampled. Seeds from each source population were maintained and tracked separately, but maternal lines within each source were not distinguished.

It is initially more costly to collect and maintain seeds from each maternal line separately than it is to make bulk collections. But, it is the only practical way to equalise and maximise the contribution of particular founding individuals to a reintroduced population. In some cases, especially where source populations are very small, it may be vitally important to maximise the effective population size of a reintroduced population. It is also more costly to follow the fates of specific individuals of known history than it is to follow the fate of populations in the aggregate. Even if maternal lines have been kept separate, it is not necessary to follow the fates of individuals. Simply by choosing equal numbers of propagules from each maternal line, it is possible to better equalise and maximise the number of founders in a reintroduced popualtion.

Reintroduction site: location, management and habitat

Questions about reintroduction site are viewed here from two perspectives. One concerns suitability of the location itself to support the reintroduced species, and includes geographic location as well as intrinsic ecological and extrinsic land-management factors. These influence the choice of a reintroduction site in the larger, geographic sense. The other concerns activities that take place at the reintroduction site itself, both with respect to observation of habitat characteristics and also any experimental manipulation of the habitat.

The choice of a suitable reintroduction site in the larger geographical sense is a complex topic, and is beyond the scope of this paper. A more comprehensive treatment by Fiedler and Laven (1996) outlined four classes of site-selection criteria. Physical criteria are relatively straightforward, and include both large-scale geomorphic properties, and finer-scale factors, such as specific soil types and other characteristics. Biological criteria include both autecological and synecological factors. Not only must the reintroduced species be able to survive and grow initially, but it has to be embedded in a larger ecological community that fosters persistence. Their other two classes of site-selection criteria, logistical and historical, are more amorphous. Logistical factors include not only such pedestrian concerns of ease of access by researchers, but also longer-term concerns of land ownership and land-use management plans. Historical site-selection criteria include whether the taxon is known to have occupied the site in the past, but also looks to the long-term future prospects of a site providing suitable habitat.

Suffice it to say that the choice of reintroduction sites is a highly complex one, and contingent on many circumstances. At a minimum, reintroduction sites should offer habitat suitable for the plant to establish and persist and appropriate historical and future land use or management. Reintroduction sites should be located on land that is legally or otherwise protected from deliberate human disturbances (i.e. conversion to other land uses, such as agriculture, or housing and so on) that would reduce or destroy its ability to support the population.

A more fine-grained view of the reintroduction site has to do with specific characteristics of the habitat itself. Many improvements to reintroduction techniques can be learned from observing the effects of different microhabitats on establishment, growth and survival of reintroduced plants at the time of planting, and later monitoring. Additional insight can be gained by experimentally manipulating habitat characteristics to examine specific hypotheses about various factors of interest (Table 1).

For example, in the *Arabis* reintroduction, observational data were gathered for each seed and transplant on slope (at two different scales, 1 dm and 1 m), aspect, and several components of planting substrate such as soil texture and existing vegetation. Because of high mortality, the results were difficult to interpret, but at least initially, it seems that habitats favouring seedling establishment may differ from those favouring establishment of transplants. Establishment from seed was better on southern aspects, whereas transplants had higher survival on south-western exposures. Also, transplants on ledges (shallow slope over 1 dm, and very steep slope over 1 m) did better than other combinations.

An example of experimental manipulation of the reintroduction habitat involves the removal or not of the ground cover at the time of planting in the *Lilium occidentale* reintroduction. The results indicate that the effect, if any, on either establishment or subsequent growth and survival resulting from removal of competing ground cover, is minor and not consistent.

The series of reintroduction projects with prairie species involved both observation of habitat characteristics and experimental manipulation (Table 1). Although the reintroduction site was superficially flat and level, subtle variations in elevation, of the order of 1–2 dm over a scale of many hundreds of metres of landscape, resulted in substantial variation in hydrology and plant community structure. All four species showed an effect of microtopographic position. *Lomatium* survived better in the lower areas in which standing water would accumulate in winter and spring, and the other three, *Seriocarpus*, *Erigeron* and *Horkelia*, had greater survival in the higher, drier microsites. Adding a slow-release fertiliser at the time of planting appeared to have a net negative effect on the growth of *Seriocarpus*, *Erigeron* and *Horkelia*. For *Lomatium*, there was a significant interaction between fertiliser and planting season, such that fertiliser increased survival of fall transplants only; without fertiliser, spring was a superior planting season.

Many species have symbiotic relationships with other organisms that may affect the survival and growth of reintroduced plants. Growth rates, survival prospects and reproductive output of many legumes can be increased in the presence of nitrogen-fixing symbiotic bacteria (*Rhizobium* spp.). Transplants of *Lupinus sulphureus* var. *kincaidii* (Fabaceae) were inoculated at planting in 2000. There was no effect of inoculation on survivorship, but after 4 years, plants given nodulating bacteria were more likely to flower than uninoculated controls (Kaye and Brandt 2005).

A different set of experiments was conducted on a species, *Castilleja levisecta*, that has become extirpated from the southern half of its historic range, from the Willamette Valley of Oregon, north through the Puget Trough and on several islands in Puget Sound, in Washington, and adjacent British Columbia.

Castilleja is a hemiparasitic plant whose roots can penetrate the roots of other plants and acquire moisture, nutrients and other compounds directly from their host. In greenhouse experiments to evaluate alternative hosts for use in reintroductions, *C. levisecta* grew larger in pots with *Eriophyllum lanatum* (Asteraceae) than with *Festuca roemeri* (Poaceae) or no host at all. However, when moved to a field site, host preference was reversed. Plants with *Festuca* had higher survival than those with *Eriophyllum* (Lawrence 2005), suggesting that caution should be used when extrapolating results of laboratory experiments to field applications.

Timing

As used here, timing issues include both the season in which seeds are sown or transplants are planted, and also the number of times reintroduction is attempted. Only in the four prairie species was planting season an experimental variable. Fall planting was superior to spring in 1 of 2 years in *Seriocarpus*, and in *Lomatium* (but in 1 year, only when fertilised). Spring was consistently superior to fall for both *Erigeron* and *Horkelia*.

We have generally not used multiple plantings in different years as an experimental variable in these studies. Nevertheless, *a priori*, the more attempts are made in different years, the greater the likelihood will be of successfully establishing new populations. Multiple seeding events have become the recommended approach for *Abronia*, because establishing a persistent seed bank appears to be crucial for successfully reintroducing this species to any given site (Kaye 2004). Single seeding events often fail after 2 years in this species.

Experimenting with timing may be necessary for developing successful reintroduction protocols. Although we cannot offer generalisations about the most appropriate season for establishing plants because of the great variability of climates and habitats around the world, it is clear that the old adage 'timing is everything' may hold true in the context of endangered-species reintroduction.

Evaluating biological and project success

Success is a problematic concept, having both short- and long-term aspects, as well as absolute and relative meanings. Biological success focuses on the performance of individuals, populations and metapopulations. Project success is a broader concept that includes the generation of new information about the reintroduced species, or reintroduction methods, or even the influence of public debate on conservation policy. Pavlik (1996) characterised the goals of rare-plant reintroduction from four vantage points, which include abundance, extent, resilience and persistence. Abundance has to do with the establishment, vegetative growth, fecundity and ultimately the population size of the reintroduced population. By extension, Pavlik (1996) broadened this scope to include dispersal, and the relationship of the reintroduced population to other populations. Resilience is the ability to survive environmental perturbations, and is enhanced by genetic diversity and seed or vegetative dormancy, or the ability to resprout after severe fires. Persistence is in a sense the summation of the others, and implies a population has become self-sustaining and functions within the ecological community. How successful have these projects been, and what has been learned in the process?

Table 3 summarises the performance of the taxa described here against a series of biological hurdles, or benchmarks of biological success. The criteria include initial establishment of the founding propagules, and their subsequent survival through a juvenile period to reproductive status, and then on to the production of a second generation. It is too early to determine whether any of these reintroduced populations will become self-sustaining, and to what degree they become resilient to environmental perturbation.

All reintroduction attempts resulted in at least some initial establishment. Least successful were the *Arabis* seedlings, which did not survive their first summer. In every other case, founding plants survived for some time as juveniles. Founders of all species except *Lilium* have reached reproductive maturity, although have not necessarily produced a second generation. Because it is an augmentation at a site where naturally occurring *Arabis* plants are found, and because seed dispersal itself was not directly observed, we cannot know whether seeds produced by transplanted individuals have germinated or become established.

Where biological failure is easily recognised, success is more elusive. It is simply too early to judge whether these projects will produce self-sustaining populations that are resilient to disturbance. Short-term measures of success do not necessarily translate into long-term success. Most of the projects here are five or fewer years old, and one, *Lilium*, has survived for a decade. After reintroduction, *Stephanomeria*, an annual, survived at the site for 15 years before becoming extinct in the wild for the second time. Never known from more than ~1000 plants in any year, over 40 000 seeds of this naturally genetically depauperate, self-pollinating species are in *ex situ* storage at the Berry Botanic Garden. With its autogamous breeding system, and because it is relatively easy to grow, and produces copious seed, a second attempt could be based on seed produced after one or more cycles of *ex situ* cultivation. Even though the original experiment involved transplants, a second attempt is in the early planning stages, and may be appropriate with the use of seeds. To the degree that the *Abronia* example provides a legitimate model to emulate, the population might be successfully re-established

Table 3. Summary results of reintroduction experiments on taxa described in text, for various measures of biological success, as of 2005

It is too early to determine whether populations are self-sustaining, and to what degree they are resilient to environmental perturbation

Taxon	Propagule type	Initial establishment	Juvenile	Reproductive	Next generation	Extant in 2005
Abronia	Seed	Yes	Yes	Yes	Yes	Yes & no
	Plant	Yes	Yes	Yes	Yes	Yes & no
Arabis	Seed	Yes	No	No	No	No (<1 year)
	Plant	Yes	Yes	Yes	Yes	Yes (4 years)
Aster	Seed	Yes	Yes	Yes	Yes	Yes (5 years)
	Plant	Yes	Yes	Yes	Yes	Yes (5 years)
Castilleja	Plant	Yes	Yes	Yes	Yes	Yes (2 years)
Erigeron	Seed	Yes	Yes	Yes	Yes	Yes (5 years)
	Plant	Yes	Yes	Yes	Yes	Yes (5 years)
Horkelia	Seed	Yes	Yes	Yes	Yes	Yes (5 years)
	Plant	Yes	Yes	Yes	Yes	Yes (5 years)
Lilium	Seed	Yes	Yes	Not yet	Not yet	Yes (10 years)
	Plant	Yes	Yes	Not yet	Not yet	Yes (10 years)
Lomatium	Seed	Yes	Yes	Yes	Yes	Yes (5 years)
	Plant	Yes	Yes	Yes	Yes	Yes (5 years)
Lupinus	Seed	Yes	Yes	Yes	Yes	Yes (5 years)
	Plant	Yes	Yes	Yes	Yes	Yes (5 years)
Stephanomeria	Plant	Yes	Yes	Yes	Yes	No (~15 years)

by simple broadcast seeding for one to a few years with large numbers of seed.

If realising biological success is problematic, project success is at least easier to recognise. Regardless of biological outcome, each of these studies yielded practical information of value concerning not only how to better reintroduce the particular species in question, but also, taken together, some tentative generalisations are beginning to emerge.

Perhaps the clearest and most consistent result across studies is that, as founders, transplants are more likely to result in greater establishment than are seeds. Differences in establishment rates range from relatively small to very large. Nevertheless, other factors need to be considered when choosing the propagule type for a project. A taxon's rarity and degree of endangerment, as well as the availability, abundance and cost of seeds and transplants, in both labour and monetary terms, will affect the decision. Where sufficient seed is available, as was the case with *Abronia*, direct seeding can be an effective method, even if establishment rates of seeds are significantly lower than those of transplants. In contrast, where seeds are limiting, the relatively greater yield per seed by transplants may outweigh the greater labour and monetary costs associated with the use of transplants.

The theoretical risks and benefits of using a sole source *v.* mixed population founders are relatively clear, at least in broad outline (e.g. Guerrant 1996*a*; Kaye 2001). These studies provided less direct insight into questions of how many and which source populations to use than they did for propagule type. This is because the actual long-term genetic impact of using mixed-source *v.* single-source founding populations in any given project may not be known for many generations, and then only with careful comparative genetic, ecological and demographic analyses.

Beyond identifying the common factors and questions common to many reintroduction projects, our goal for this paper was to demonstrate through examples that reintroductions are best served by an experimental approach. The benefits are 3-fold. First, comparison of different techniques and incorporation of observational data can result in improvements to reintroduction protocols for any given species. Second, the use of more than one method increases the likelihood that the reintroduction will be successful, at least in terms of numbers of established plants. Put another way, trying more than one approach at a time can be viewed as a bet-hedging strategy in case any single method fails. Finally, endangered-plant reintroduction is still a relatively new field, and we argue that an experimental approach, either with case studies or more far-reaching tests, is necessary to build the literature base needed for developing generalisations and theory.

Because these projects were set up as scientific experiments designed to test specific hypotheses, they have all provided considerable information about particular species and reintroduction sites. The manipulative experiments outlined here have been useful for making recommendations for further reintroduction projects with these plants. For example, identifying the appropriate season to plant, as well as whether or not to use a fertiliser, may improve future outplantings. *Erigeron*, in particular, has a much higher survival when planted in the spring without fertiliser (48% after 4 years), but survival of fall plantings with added nutrients was very low (3%). The difference here is so substantial that it could mean the difference between success and failure of a future project.

Observational studies can also yield significant findings and, in some cases, may be easier than controlled experiments. Measuring the effects of different habitat characteristics on plant establishment, survival and growth can be straightforward and informative. It is noteworthy that in *Arabis* and *Lilium*, observations of microsite habitat may have provided more information useful for understanding which habitat characteristics foster establishment and survival, than did manipulation, which both entailed greater resources be used, while not necessarily improving survival prospects.

Acknowledgements

We are grateful to the many colleagues who have assisted us in our work, some for many years. In particular EOG thanks Andrea Raven and the many interns and volunteers of the Berry Botanic Garden, and Bruce Rittenhouse and Nancy Brian of the Salem District, and Russ Holmes and Susan Carter of the Roseburg District, USDI Bureau of Land Management. TNK gratefully acknowledges Beth Lawrence, Jen Cramer, Angela Brandt, and other staff of the Institute for Applied Ecology, as well as Nancy Sawtelle and Sally Villegas of the Eugene District, USDI Bureau of Land Management. We also thank three anonymous reviewers for their constructive suggestions. We also gratefully acknowledge many years of funding and other logistical support by the USDI Bureau of Land Management and USDA Forest Service, which made many of these projects possible. Finally, Dr David Coates, and the other organisers of the symposium in which a version of this paper was delivered by EOG, have our thanks, as does the Department of Conservation and Land Management for financial and other support that made our participation possible.

References

Borlase SC, Loebel DA, Frankham R, Nurthen RK, Briscoe DA, Daggard GE (1993) Modeling problems in conservation genetics using captive *Drosophila* populations: consequences of equlization of family sizes. *Conservation Biology* **7**, 122–131. doi: 10.1046/j.1523-1739.1993.07010122.x

Falk D, Millar CI, Olwell M (Eds) (1996) 'Restoring diversity: strategies for reintroduction of endangered plants.' (Island Press: Washington, DC)

Fiedler PL, Laven RD (1996) Selecting reintroduction sites. In 'Restoring diversity: strategies for the reintroduction of endangered plants'. (Eds DA Falk, CI Millar, M Olwell) pp. 157–169. (Island Press: Washington, DC)

Guerrant EO Jr (1996*a*) Designing populations: demographic, genetic, and horticultural dimensions. In. 'Restoring diversity: strategies for reintroduction of endangered plants'. (Eds DA Falk, CI Millar, M Olwell) pp. 171–207 (Island Press: Washington, DC)

Guerrant EO Jr (1996*b*) Reintroduction of *Stephanomeria malheurensis*, a case study. In 'Restoring diversity: strategies for reintroduction of endangered plants'. (Eds DA Falk, CI Millar, M Olwell) pp. 399–402. (Island Press: Washington, DC)

Guerrant EO Jr (2001) Experimental reintroduction of the endangered western lily (*Lilium occidentale*). In 'Conservation of Washington's native plants and ecosystems'. (Eds SH Reichard, PW Dunwiddie, JG Gamon, AR Kruckeberg, DL Salstrom) pp. 201–211. (Washington Native Plant Society: Seattle)

Guerrant EO Jr (2005*a*) Reintroduction (Augmentation) of Koehler's rockcress (*Arabis koehleri* var. *koehleri*) at the BLM North Bank Habitat Management Area: Survival and growth during the three years after planting. Report prepared for the USDI Bureau of Land Management, Roseburg District, Roseburg, OR.

Guerrant EO Jr (2005*b*) Experimental reintroduction of western lily (*Lilium occidentale*) at the New River ACEC: Results of the first eight years of growth. Report prepared for the USDI Bureau of Land Management, Coos Bay District, North Bend, OR.

Guerrant EO Jr, Pavlik BM (1997) Reintroduction of rare plants: genetics, demography and the role of *ex situ* conservation methods. In 'Conservation biology for the coming decade'. 2nd edn. (Eds PL Fiedler, P Kareiva) pp. 80–108. (Chapman and Hall: New York)

Guerrant EO Jr, Havens K, Maunder M (Eds) (2004) '*Ex situ* plant conservation: supporting species survival in the wild.' (Island Press: Covelo)

Kaye TN (2001) Common ground and controversy in native plant restoration: the SOMS debate, source distance, plant selections, and a restoration-oriented definition of native. In 'Native plant propagation and restoration strategies'. (Eds R Rose, D Haase) (Nursery Technology Cooperative and Western Forestry and Conservation Association: Corvallis)

Kaye TN (2003) Experimental reintroduction of pink sandverbena on the Oregon coast: transplanting and seeding. Unpublished technical report prepared for the USDA Forest Service, Siuslaw National Forest, and USDI Bureau of Land Management, Coos Bay District.

Kaye TN (2004) Reintroducing the endangered pink sand-verbena to Pacific Coast beaches: Direct seeding and out-planting. In 'The ecology and management of rare plants of northern California'. (Eds MB Brooks, LK Carothers, T LaBanca) pp. 131–139. (California Native Plant Society: Sacramento, CA)

Kaye TN, Brandt A (2005) Seeding and transplanting rare Willamette Valley Prairie plants for population restoration. Unpublished technical report prepared for the Eugene District, USDI Bureau of Land Management.

Kaye TN, Cramer JR (2003) Direct seeding or transplanting: the cost of restoring populations of Kincaid's lupine (Oregon). *Ecological Restoration* **21**, 224–225.

Kaye TN, Lawrence BA (2003) Fitness effects of inbreeding and outbreeding on golden paintbrush (*Castilleja levisecta*): implications for recovery and reintroduction. Institute for Applied Ecology, Corvallis, Oregon, and Washington Department of Natural Resources, Olympia, Washington.

Lawrence BA (2005) Studies to facilitate reintroduction of golden paintbrush (*Castilleja levisecta*) to the Willamette Valley, Oregon. Masters Thesis, Oregon State University, Corvallis, OR.

Lawrence BA, Kaye TN (2006) Habitat variation throughout the historic range of golden paintbrush, a Pacific Northwest prairie endemic: Implications for reintroduction. *Northwest Science* **80**, 140–152.

Loebel DA, Nurthen RK, Frankham R, Briscoe DA, Craven D (1992) Modeling problems in conservation genetics using captive *Drosophila* populations: consequences of equalizing founder representation. *Zoo Biology* **11**, 319–332. doi: 10.1002/zoo.1430110504

McGlaughlin M, Karoly K, Kaye TN (2002) Genetic variation and its relationship to population size in reintroduced populations of the pink sand verbena, *Abronia umbellata* subsp. *breviflora. Conservation Genetics* **3**, 411–420. doi: 10.1023/A:1020507416654

Menges ES, Guerrant EO Jr, Hamzé S (2004) Effects of seed collection on the extinction risk of perennial plants. In *ex situ* plant conservation: supporting species survival in the wild. (Eds EO Guerrant Jr, K Havens, M Maunder) pp. 305–324. (Island Press: Washington, DC)

Newman D, Pilson D (1997) Increased probability of extinction due to decrease genetic effective population size: experimental populations of *Clarkia pulchella. Evolution* **51**, 354–362. doi: 10.2307/2411107

Pavlik BM (1996) Defining and measuring success. In 'Restoring diversity: strategies for the reintroduction of endangered plants'. (Eds DA Falk, CI Millar, M Olwell) pp. 127–155. (Island Press: Washington, DC)

Parenti RL, Guerrant EO Jr (1990) Down but not out: reintroduction of the extirpated Malheur wirelettuce, *Stephanomeria malheurensis. Endangered Species UPDATE* **8**, 62–63.

Manuscript received 20 February 2006, accepted 9 October 2006