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Plant ecotypes: genetic differentiation in the age of ecological restoration

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Recent studies illustrate the emerging field of restoration genetics, which is a synthesis of restoration ecology and population genetics. The translocation of organisms during the restoration of native ecosystems has provoked new questions concerning the consequences of sampling protocols and of intraspecific hybridization between locally adapted and transplanted genotypes. Studies are now underway to determine both the extent of local adaptation among focal populations and the potential risks of introducing foreign genotypes, including founder effects, genetic swamping and outbreeding depression. Data are needed to delineate 'seed transfer zones', or regions within which plants can be moved with little or no consequences for population fitness. Here, we address the revival of transplant and common garden studies, the use of novel molecular markers to predict population genetic consequences of translocation, and their combined power for determining appropriate seed transfer zones in restoration planning for native plant populations.

The preservation of native plant communities must include their restoration if land managers and conservation biologists are to counter the pressures of urban development and the threat of invasive exotic species. However, restoration ecology is a relatively young science and few criteria exist for the creation of self-sustaining populations that retain adaptive genetic variation. Specifically, ecological restoration of native plant species often requires seed stock to replace or augment threatened plant communities. Seeds derived from different sources might represent genetically novel or depauperate material because of the combination of restricted gene flow among populations, adaptation to local (see Glossary) environmental conditions and limited seed collections.

The geographical distribution of many plant species included in restoration efforts spans a wide range of climatic and edaphic conditions. Habitat heterogeneity, combined with natural selection, often results in multiple, genetically distinct ecotypes within a single species [1–3]. Given the potential for introducing genotypes that are poorly adapted to the site of restoration, studies of local adaptation assume new importance if we aim to restore population fitness as well as increase the abundance of native plant species. This awareness, combined with recent studies of the negative consequences of outbreeding, has fueled new efforts to understand the effects of large-scale introductions on population mean fitness [4–10].

Here, we review field and greenhouse studies that have direct implications for the effects of translocation on plant community restoration, particularly those resulting from intraspecific hybridization. Moreover, we aim to clarify the differences among alternative genetic phenomena that might result in the reduced performance of first- (F1) and/or second-generation (F2) hybrids. Distinguishing among the alternative causes of such outbreeding depression could, in turn, assist in the identification of populations between which seeds might be transferred

Glossary

Co-adapted gene complexes: particular combinations of genes at multiple loci that interact to confer higher fitness relative to other genotypes.

Cryptic invasion: an undetected increase in frequency of foreign genotypes following introduction of genetic variants of the same species or of a closely related congener. Results in genetic swamping of native genotypes.

Dilution: reduction in fitness of hybrids relative to parents caused by expression of only one half of locally adapted alleles. The heterozygous hybrids are underdominant relative to the performance of each parental population in its home environment. Also known as the 'ecological' or 'environmental' mechanism of outbreeding depression.

Ecotypes: distinct genotypes (or populations) within a species, resulting from adaptation to local environmental conditions; capable of interbreeding with other ecotypes or epitypes of the same species.

Epitypes: distinct genotypes (or populations) within a species, resulting from adaptation to a specific (local) genetic background; capable of interbreeding with other epitypes or ecotypes of the same species.

Genetic swamping: rapid increase in frequency of an introduced genotype (or introduced allele) that might lead to replacement of local genotypes; caused by a numerical and/or fitness advantage.

Home-site advantage: fitness advantage of local genotypes (ecotypes) relative to introduced genotypes (ecotypes).

Hybrid breakdown: reduction in fitness of hybrids relative to parents caused by disruption of co-adapted gene complexes via recombination. Hybrid breakdown might not occur until the F2 and subsequent generations. Also known as the 'physiological' or 'genetic' mechanism of outbreeding depression.

Intraspecific hybridization: mating between individuals from genetically distinct populations of the same species.

Introduced: genotype moved into a new site. Common synonyms include 'alien', 'foreign' and 'nonlocal'.

Local: previously existing genotype at a site. Common synonyms include 'existing', 'extant', 'indigenous' and 'native'.

Local adaptation: process by which populations genetically diverge in response to natural selection specific to their habitat.

Outbreeding depression: reduction in mean population fitness resulting from hybridization between genetically distinct individuals or populations of the same species; detected in F1 or subsequent generations.

Seed transfer zones: geographical regions within which individuals (seeds, seedlings, or adults) of native species can be transferred with no detrimental effects on population mean fitness.

Translocation: a deliberate or accidental movement of species by humans that 'includes reintroduction, introduction, relocation, re-enforcement, supplementation, restocking and other synonymous terms' [4].

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with low risks of fitness reduction following hybridization. Ideally, 'restoration genetics' combines familiar common garden and reciprocal transplant studies with the use of molecular markers to detect gene flow and hybridization. Estimates of local adaptation, gene flow among conspecific populations, gene flow following translocations and the fitness consequences of intraspecific hybridization contribute critically to the delineation of SEED TRANSFER ZONES used for the collection of seed or vegetative stock in restoration.

Population genetic effects of translocation

Founder effects

Founder effects are likely to occur if seeds used to revegetate restoration sites are collected from a limited number of sources. Similar to episodes of colonization, the 'founding' propagules can represent only a portion of the allelic diversity present in the source populations, and they might hybridize with local genotypes. Resulting genetic bottlenecks can be severe if the local population is no longer present at the restoration site and the INTRODUCED population size is small [11]. Ultimately, levels of genetic variation and inbreeding in the restored population will be determined by the number and genetic diversity of founders, the mating system and the population growth rate [12].

Two recent examples illustrate the role of founder effects in restoration efforts. Williams and Davis [13] compared levels of genetic diversity of restored eelgrass *Zostera marina* populations with levels observed in undisturbed populations. Molecular analyses revealed significantly lower genetic diversity in transplanted eelgrass beds than in natural beds, and a subsequent study [14] determined that the genetic bottleneck was caused by collection protocols. Moreover, the loss of genetic variation corresponded to lower rates of seed germination and fewer reproductive shoots, suggesting that there might be long-term detrimental effects for population fitness [14].

In a second example, an investigation of population genetic integrity following the re-introduction of the endangered Mauna Kea silversword *Argyroxiphium sandwicense* ssp. *sandwicense* determined that the restored population of ~1500 plants is descended from only two or three founders [15,16]. As a result of limited seed collections, the restored population suffered a severe genetic bottleneck, the fitness consequences of which are not yet documented (because of the long-lived, predominantly monocarpic character of the species). Efforts to increase the number of founders are reportedly underway [15,17].

Genetic swamping

If remnants of the focal species are present at the restoration site, translocation of native species during restoration might result in the GENETIC SWAMPING of local genotypes [18–20]. Swamping can occur in the absence of intraspecific hybridization because of either a numerical or fitness advantage of introduced plants. Alternatively, swamping might result from the introgression of genes of introduced plants through hybridization with the local population.

For example, a recent study of common reed *Phragmites australis* determined that the current, rapid expansion of this species in North America originated with the introduction of a genetic variant from Eurasia in the early 1900s [20]. Although *P. australis* is native to North America, the spread of the aggressive genotype represents a CRYPTIC INVASION that was detected via molecular analysis of chloroplast DNA haplotypes [20,21]. The expansion coincides with a loss of genetic diversity as historic North American genotypes are swamped by the aggressive strain. *Phragmites australis* spreads primarily through vegetative growth and its range expansion corresponds to genetic swamping with no reported intraspecific hybridization [22].

Swamping via intraspecific hybridization as a consequence of plant species restoration has not been studied. Instead, research has focused on interspecific hybridization and its consequences for the conservation of native populations [23–25]. Genetic swamping of local genotypes can occur by hybridization between two congeners, as has occurred between the native California cordgrass *Spartina foliosa* and the introduced *S. alterniflora*. Anttila *et al.* [26] discovered that rare F1 hybrids of the two species are highly vigorous and threaten the genetic integrity of the native plant populations because of enhanced pollen production and dispersal. This phenomenon can also be expected when genetically distinct conspecific populations hybridize, but it is relatively difficult to detect. To document swamping within a single species, molecular analyses of local and introduced plants must occur before and after the translocation.

Heterosis and outbreeding depression

When there are remnant populations at a restoration site, translocation of conspecifics with compatible and phenologically synchronous mating systems can result in intraspecific hybridization. The fitness of hybrids (grown in the habitat of the local parent) relative to their parents will depend on the underlying cause of genetic differentiation among parental populations. Consequences of intraspecific hybridization might include heterosis and/or outbreeding depression.

If population divergence is caused by genetic drift, hybridization often results in heterosis, or increased vigor of F1 hybrid progeny [8,27–32]. Heterosis occurs when hybrid fitness is enhanced relative to the local parental population and is caused by either the masking of recessive deleterious alleles or by an overall fitness advantage of heterozygotes (overdominance) [30,33]. Multilocus mechanisms that account for the higher performance of hybrids include the combination of favorable dominant alleles fixed at different loci in parental populations, and the generation of novel and favorable multilocus genotypes (epistasis) [33].

Alternatively, hybridization between individuals from genetically distinct populations can result in the lower fitness of hybrids through outbreeding depression. Two proposed genetic mechanisms that underlie outbreeding depression are DILUTION and HYBRID BREAKDOWN (Box 1).

Box 1. Mechanisms of outbreeding depression

Outbreeding depression is the reduction in fitness of individuals resulting from crosses between genetically distinct parents (e.g. intraspecific hybridization), and is caused by one of two mechanisms. The first mechanism is the ‘dilution’ of locally adapted genotypes following hybridization between populations that have diverged because of natural selection, and which have become fixed for different alleles [7,9,62]. F1 hybrids are heterozygous at locally adapted loci, resulting in the 50% ‘dilution’ of each differently adapted, parental genome. The resulting underdominance (where the hybrid performs worse than either parental genotype) is expressed in F1 hybrids raised in the native environments of both parents.

The second mechanism is ‘hybrid breakdown’ through the loss of co-adapted gene complexes or ‘intrinsic co-adaptation’ [7,34,62,63]. Hybrid breakdown results from the recombination or ‘shuffling’ of adaptive, multi-locus gene combinations during sexual reproduction, and might not be expressed until the F2 generation or later. This one-generation delay is explained by the presence in F1 hybrids of two intact, adaptive multilocus gene combinations (one from each parent) that later ‘break down’ in the second generation when recombination first occurs [33,62–64]. Ultimately, hybrid breakdown is the reduction in fitness associated with the disruption of epistatic interactions among loci.

Whereas dilution is a consequence of local adaptation, hybrid breakdown can result from hybridization between populations that have diverged in one of two ways [34]. First, hybrid breakdown can result from mating among genotypes adapted to distinct environments. Second, hybrid breakdown can result from mating among genotypes with distinct combinations of epistatically interacting loci (CO-ADAPTED GENE COMPLEXES). In the second case, populations do not represent ecotypes adapted to either the biotic or abiotic environment, but instead have become differentiated by genetic drift followed by selection for alleles favored within each population-specific genetic background [35,36].

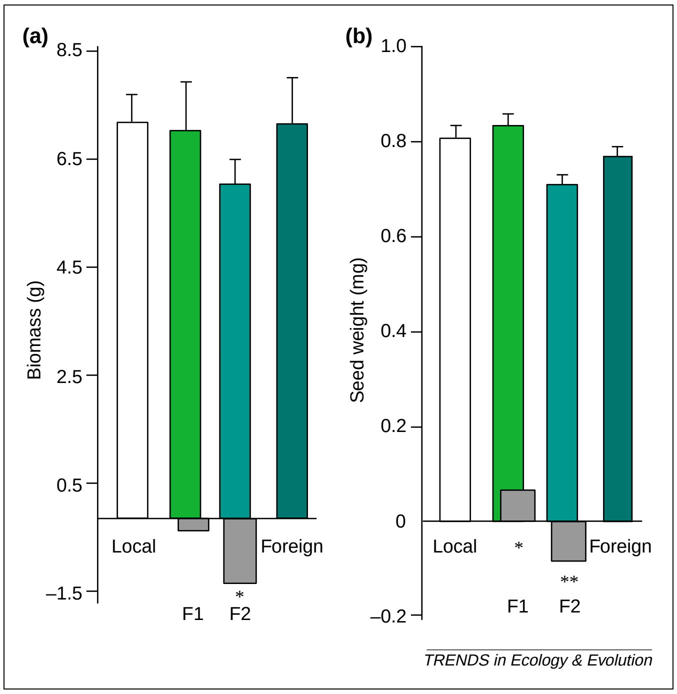

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Fig. 1. Examples of heterosis and/or outbreeding depression following hybridization between local and foreign genotypes raised under common field conditions. Gray bars represent deviations (of the corresponding green bar) from the expected values assuming no heterosis or epistasis (*$P < 0.05$; ** $P < 0.01$). (a) Mean phenotypes for shoot biomass of parental genotypes and F1 and F2 hybrids of *Agrostemma githago* raised in Switzerland. ‘Local’ and ‘Foreign’ parental genotypes were sampled from Switzerland and central Germany, respectively. The F2 hybrids were produced by backcrossing maternal Swiss genotypes to paternal F1 hybrids. The mean biomass of the F1 hybrids did not differ significantly from that expected in the absence of heterosis. The mean biomass of the F2 hybrids was significantly lower than that expected, revealing hybrid breakdown. (b) Mean phenotypes for seed weight of parental genotypes and F1 and F2 hybrids of *Silene alba* raised in Switzerland. ‘Local’ and ‘Foreign’ parental genotypes were sampled from Switzerland and Hungary, respectively, and crossed to produce F1 and F2 progeny as in (a). The F1 hybrids exhibit heterotic effects on seed mass, whereas the F2 hybrids exhibit outbreeding depression; F2 seed mass was lower than that expected when assuming no epistasis. Adapted, with permission, from [8].

We distinguish between the two cases of hybrid breakdown by contrasting ecotypes with EPITYPES (a new term introduced here and distinct from the same term used in botanical nomenclature). In the simplest cases, where restoration efforts combine different ecotypes, F1 hybrid fitness should be intermediate between the two parents observed at the local parents’ site. Where the parent populations represent epitypes, F1 or subsequent hybrid fitness might be lower than that of either parent observed in the habitat of the local parents (Box 2).

Experimental studies of intraspecific hybridization

Recent studies of heterosis and outbreeding depression in plant species are summarized in Table 1. To date, most research examines the fitness consequences of intraspecific hybridization by comparing parents to F1 hybrid progeny [10,27–29]. However, there is a growing effort to contrast the performance of F2 (and later) hybrid progeny as well as F1 hybrids with parental genotypes [7,8,30]. These studies reveal a pattern of F1 hybrid heterosis followed by outbreeding depression expressed in later generations. An intermediate crossing distance advantage is also often observed in F1 hybrids [37,38]. This is explained by the expression of inbreeding depression following crosses between near neighbors and outbreeding depression following crosses between distant parents. However, patterns of hybrid fitness are not consistent among all traits or taxa. Similar to scenarios presented in Box 2, some results suggest heterosis alone regardless of outcrossing distance [27,29] whereas others detect only outbreeding depression [10].

Among these studies of intraspecific hybridization, an increasing number are being conducted in the context of ecological restoration [7,8,10]. This new emphasis follows the realization that introgression of maladapted genotypes after translocation can threaten the long-term sustainability of restored populations. Keller *et al.* [8] examined fitness effects of hybridization among introduced and local populations of three species used in wildflower reseeding of Swiss agricultural field margins. Results for two fitness traits (shoot biomass and seed mass) in two species are shown in Fig. 1. Other combinations of heterosis and outbreeding depression have been found in *Chamaecrista fasiculata* and *Lotus scoparius* (Table 1), indicating a need for more research to determine the patterns of outbreeding depression that are relevant to restoration [7,10].

Table 1. Studies to detect outbreeding depression among geographically isolated populations of the same species[a]

Species	No. of populations used in crosses/crossing dist.	Hybrid gen.	Performance of F1 generations	Performance of F2 and later generations	Refs
Arnica montana	5 pop.; inter- and intrapop. crosses	F1	Heterosis for total seed number per plant, and for probability of flowering by adults	N/A	[32]
Delphinium nelsonii	155 plants in 1 pop.; dist. classes: 1 m, 3 m, 10 m and 30 m	F1	Inbreeding and outbreeding depression observed for growth and survival Progeny of intermediate distance crosses performed 5–8 times better than did progeny of 30-m cross	N/A	[37]
Eupatorium resinosum	2 pop. (1-way crosses); dist. classes: near neighbors, distant neighbors within pop., and between pop.	F1	Plant size and reproduction increased with crossing distance Near-neighbor crosses produced less fit progeny than did distant-neighbor and between-pop. crosses	N/A	[28]
Gentiana pneumonanthe	58 plants in 1 pop.; dist. classes: self, 1–50 cm, 50–100 cm, 1–2 m, 2–5 m, 5–10 m, 10–20 m, &greater;20 m and 800 m	F1	No effect of pollen donor dist. if selfing and interpop. crosses excluded Heterosis observed for seed set Inbreeding depression observed for seed mass and adult biomass	N/A	[27]
Ipomopsis aggregata	Partial diallel breeding design using 60 parents; dist. classes: 1 m, 10 m and 100 m	F1	Significantly greater seedling emergence for 1-m and 10-m progeny compared with 100-m progeny Optimal outcrossing distance observed; progeny from 10-m crosses outperformed progeny from 1-m and 100-m crosses for estimates of lifetime fitness, but differences not signif.	N/A	[38]
Lotus scoparius	6 pop.; 80–400 km apart	F1	Outbreeding depression following pollinations (seeds/flower × seedlings/seed); magnitude positively correlated with genetic distance among pop. Survival x fruit production increased with mean environmental dist. among pop.	N/A	[10]
Sarracenia flava	6 pop.; 4 dist. classes or pollination treatments: control, self-pollination, intrasite cross and intersite cross	F1	Greater seed set, seed mass, germination, survivorship and growth observed in most years for intra- and intersite crosses relative to selfs Seed mass and seedling vigor greater for inter- than for intrasite crosses	N/A	[31]
Silene nutans	2 pop. 30 km apart; dist. classes: selfing, outcrossing within, and between pop.	F1	Performed as well or better than parents, but effect of distant pollen source depended on direction of interpop. Cross	N/A	[29]
Agrostemma githa	7–8 pop. min.; 4 European countries	F1,F2	Heterosis observed for seed mass and, in some cases, for shoot biomass	Strong hybrid breakdown observed for shoot biomass	[8]
Chamaecrista fasciculate	10–15 maternal families per pop.; 3 target pop.; dist. classes: 0.1 km, 1 km, 10 km, 100 km and 1000s of km	F1,F2,F3	Heterosis observed for germination, survivorship and vegetative biomass Pattern of heterosis consistent with drift being largely responsible for genetic divergence among pop.	Hybrid breakdown in F2 fruit production 5/6 cases showed hybrid breakdown in F3 (germination, biomass and fruit production) Greater hybrid breakdown in F3 suggests outbreeding depression increases with additional recombination	[30]
	5 pop. in each of 3 regions; dist. classes: 0.1 km, 1 km, 10 km, 100 km and 1000s km	F1,F3	Heterosis observed for germination, final biomass, survivorship and fruit production	F3 fitness often equal to parents for same traits observed in F1	[7]

Table 1 (*continued*)

Species	No. of populations used in crosses/crossing dist.	Hybrid gen.	Performance of F1 generations	Performance of F2 and later generations	Refs
			Strong effects of crossing distance, year and environment on hybrid breakdowm	Reduced fitness in F3 vs. F1 because of loss of heterozygosity and co-adapted gene complexes Strong effects of crossing distance, year and environment on expression of hybrid breakdown	
Papaver rhoeas	7–8 pop. min.; 4 European countries	F1,F2	Slight outbreeding depression observed for survival Trend of reduced fitness observed for hybrid progeny	Hybrid breakdown observed for survival Some evidence of hybrid breakdown observed for shoot biomass	[8]
Silene alba	7–8 pop. min.; 4 European countries and 1 USA location	F1,F2	Heterosis observed for seed mass and shoot biomass	Hybrid breakdown observed for seed mass and shoot biomass, but depended on cross and trait observed	[8]
			Reduced fitness observed in most distant cross (Swiss × USA)	No hybrid breakdown in Swiss × Hungarian cross	

[a]Studies not consistent with respect to traits examined, but all kinds of traits can show evidence of heterosis and/or outbreeding depression. More studies are needed of F2 and subsequent generations to determine whether F1 heterosis accurately reflects long-term effects on population mean fitness of intraspecific hybridization.

Alternate outcomes of intraspecific hybridization are likely to reflect the number of hybrid generations studied, crossing distances and underlying spatial environmental heterogeneity, the life-history traits examined, the degree of population inbreeding, different mechanisms of outbreeding depression and the degree of linkage (and subsequent time for disruption) of co-adapted loci.

Detecting local adaptation and risks of translocation

If researchers are to predict the population genetic consequences of translocation, estimates are needed of ecotypic and epitypic differentiation and of the likelihood of hybridization, all of which increase the potential for outbreeding depression. Here, we discuss the resurgence of classic approaches (common garden and reciprocal transplant studies) and identify applications of molecular marker assays that are relevant to restoration genetics. Each method addresses factors that are important for our understanding of the effects of interpopulation seed transfer on population mean fitness.

Common garden studies

Variation in morphology and life history within species is significant for restoration only in cases where population divergence has a genetic basis. The classic method of determining whether observed differences among populations are genetically based is the common garden study [1,2,39]. In recent studies of perennial grass species subject to restoration, common gardens were established that represented seven or more populations and were then used to detect intraspecific genetic variation [40–42]. In each case, significant differences among populations for quantitative traits were observed in the common environment. The discovery of such heritable variation suggests that caution is needed in seed transfers between dissimilar populations of these grasses. However, common garden studies do not distinguish between divergence caused by drift or natural selection.

Reciprocal transplants of parental genotypes

Reciprocal transplants among sites make it possible to determine whether population divergence represents an adaptive response to natural selection [39]. Adaptive differences between populations are detected as a HOME-SITE ADVANTAGE, whereby each genotype (i.e. ecotype) performs best at its native site [9,43]. Given the potential for the disruption of local adaptation by translocation, investigators have tested the home-site advantage hypothesis in several plant taxa used in restoration [9,41,44,45]. Strong evidence of local adaptation was discovered in 11 out of 13 species, suggesting that interpopulation transplants would suffer significantly reduced fitness at transplant sites [9,45,46]. However, there might be translocation risks even in the absence of local adaptation, when hybridizing populations represent epitypes rather than ecotypes. If epitypic differentiation is present, parental genotypes might do equally well in either parental environment, and a decline in population mean fitness would not occur until recombination disrupts co-adapted gene complexes.

Relative performance of hybrids and parents

The approaches described above can also be used to detect heterosis and outbreeding depression following intraspecific hybridization. Heterosis is detected as a fitness advantage of F1 hybrids, whereas outbreeding depression is detected as a fitness reduction of F1 (or later) hybrids, when compared with either parental population. Distinguishing between the mechanisms of outbreeding depression requires the comparison of F1 and F2 (or later) hybrid progeny raised in the same environment to determine the timing of reduced fitness (Box 2). Using these methods, researchers have discovered evidence for dilution in intraspecific F1 hybrids of *Delphinium nelsonii* and *Lotus scoparius*, and hybrid breakdown in later generations of *Chamaecrista fasiculata* [10,30,37].

Box 2. Fitness effects of intraspecific hybridization

Heterosis and outbreeding depression can have separate or combined effects on the fitness of intraspecific hybrids. Figure I depicts four potential qualitative outcomes of intraspecific hybridization on population mean fitness that have been observed in natural or experimental populations. Each graph illustrates the population mean fitness for the original, local population (dark-green bars) relative to two generations of hybrids (light-green bars) and the total population (hybrids and parental genotypes combined; red bars) following a single translocation event. Other combinations and intensities of heterosis, underdominance and hybrid breakdown would affect the short- and long-term effects of hybridization on population mean fitness. A thorough review of the underlying genetic interactions (e.g. additivity, dominance and epistasis) that might cause either enhanced or reduced hybrid fitness is beyond the scope of this article, but detailed discussions can be found elsewhere [30,33,36].

(a) Heterosis alone

Hybrid vigor might result when hybridization occurs between individuals from populations that are genetically depauperate because of drift and inbreeding (Fig. Ia). In this case, increased heterozygosity after one generation of hybridization would result in higher fitness of F1 hybrid progeny [8,27,29,31]. The relative mean fitness of the total population will also increase, but to a lesser extent, because heterosis is only apparent in hybrid individuals. Heterozygosity will decline in subsequent generations as mating (or self-fertilization) reconstitutes homozygotes among F1 progeny.

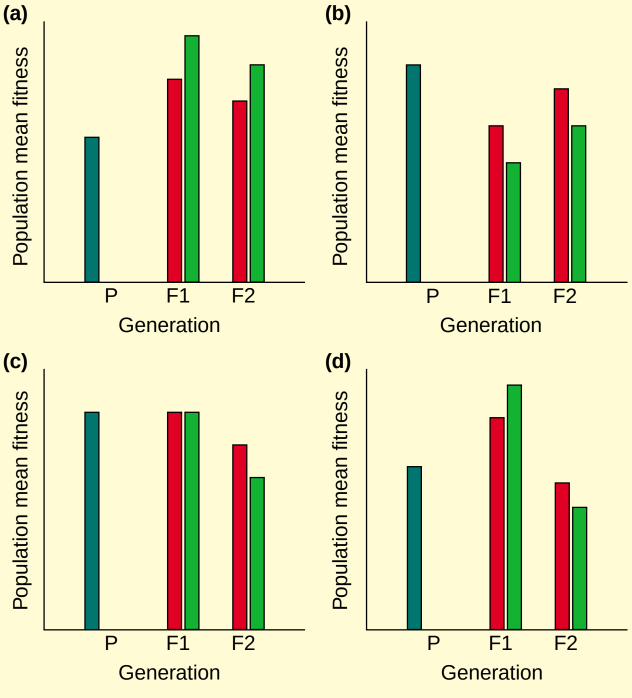

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Fig. I.

(b) Dilution (underdominance)

If introduced and local genotypes are adapted to different environments, the fitness of hybrid progeny could decrease because of a 50% dilution of the genome of the local population following hybridization [10,37] (Fig. Ib). In this case, outbreeding depression would be expressed in F1 hybrid progeny because of underdominance at loci formerly fixed for adapted alleles. Mating among the F1 progeny (or backcrossing) in the absence of further introductions would result in increased fitness of both hybrids and the total population in the F2 generation as high-fitness homozygotes are reconstituted. Recovery of population fitness would depend on the strength of local adaptation, the intensity of selection against hybrids, and the time required to restore homozygosity at loci adapted to the local environment.

(c) Hybrid breakdown alone

Hybrid breakdown might be the sole outcome of intraspecific hybridization between genotypes (Fig. Ic). Assuming that no other genetic interactions are operating (e.g. dominance or heterosis), we expect no change in population mean fitness among the F1 progeny because these hybrids contain both intact multilocus gene combinations. In the F2 generation, however, total population mean fitness and the mean fitness of hybrids will decline as co-adapted gene complexes are disrupted by recombination (there is some evidence for this in *Papaver* and *Silene* [8]). Reduced fitness caused by hybrid breakdown might occur regardless of whether the parent populations represent ecotypes or epitypes.

(d) Heterosis and hybrid breakdown present

Both heterosis and hybrid breakdown might occur after intraspecific hybridization (Fig. Id). In this case, the initial outcome of hybridization between local and introduced genotypes is hybrid vigor caused by increased heterozygosity. As a result, the total population mean fitness would also increase. However, recombination in the subsequent F2 generation would disrupt co-adapted gene complexes and counteract the increase in fitness caused by heterosis. Both F2 hybrids and the total population would suffer a corresponding loss of fitness. The severity of fitness effects in the F2 generation would depend on the joint effects of heterosis and hybrid breakdown. In addition, strong linkage between co-adapted loci might delay fitness costs until the F3 generation or later [30]. The combination of heterosis followed by hybrid breakdown in the F2 generation was found in *Agrostemma githa* and *Silena alba* [8], whereas hybrid breakdown was delayed until the F3 generation in *Chamaecrista fasciculata* [7].

Molecular marker assays

There is no strong consensus regarding how best to use molecular markers in studies of local adaptation or restoration. If molecular markers could easily detect locally adaptive traits, then labor-intensive reciprocal transplant experiments would not be necessary to detect ecotypic differentiation. There is inconsistent evidence, however, that this is the case [46–49]. Molecular markers (e.g. allozymes, amplified fragment length polymorphisms and microsatellites) often represent neutral genetic variation within and among populations, which does not necessarily correspond to adaptive variation [46,47].

Nevertheless, molecular markers are very useful for detecting three phenomena that either predict or reflect population genetic risks of restoration: (1) strong founder effects; (2) genetic swamping; and (3) population genetic divergence that might indicate ecotypic or epitypic variation [16,20,50,51]. Several recent studies serve as models for these applications of molecular markers in restoration genetics. For example, co-dominant nuclear marker analyses of *A. sandwicense* and clover *Trifolium amoenum* populations estimated small numbers of founding individuals and/or detected a loss of genetic variation as a consequence of restoration protocols [16,52].

Box 3. Future directions

There is an immediate need for additional research to describe the genetic consequences of translocation of plant species in the context of ecological restoration. In addition, these studies will improve basic understanding of the occurrence and frequency of different mechanisms of outbreeding depression. Specifically, future directions should include the following.

Effects of outbreeding depression on long-term population persistence

Future studies should evaluate the degree to which natural selection effectively eliminates poorly adapted genotypes resulting from intraspecific hybridization. This will help determine the best restoration practices (such as the use of composite mixtures of genotypes versus the use of local genotypes) when augmenting populations [59,65–67]. In addition, the presence of hybrids that fail to reproduce may reduce effective population size, threatening sustainability [68].

Timing of outbreeding depression

Given the potential for outbreeding depression in F2 or later hybrid progeny, more studies of multiple generations of hybrids are needed to document the occurrence and frequency of outbreeding depression among plant species. We also encourage accurate records of restoration practices so that plant ecologists or managers of mixed populations can return to restored sites and monitor long-term population fitness (e.g. survival and fecundity).

Predictability of intraspecific hybridization and outbreeding depression

More research that describes the expression of outbreeding depression in multiple species might help us to anticipate the attributes of species most likely to exhibit this problem. For example, highly inbred taxa are more likely than highly outcrossing taxa to evolve epitypic or ecotypic differentiation, and therefore are probably at higher risk for outbreeding depression [11]. By contrast, intraspecific hybridization is less likely to occur in inbreeding taxa.

Local adaptation in species targeted for restoration

Studies should evaluate potential source populations to determine whether they represent ecotypes or epitypes, with the goal of delineating appropriate seed transfer zones for single species or groups of species.

Uniparentally inherited markers revealed cryptic invasion via swamping by an aggressive genotype in *P. australis* [20]. Finally, molecular analyses have detected strong population differentiation in North American grasses, suggesting low levels of gene flow and potential ecotypic (or epitypic) divergence among populations [42, 50]. These results imply that translocation among these populations can result in outbreeding depression, but such marker-based predictions are best combined with field studies to confirm that genetic differentiation reflects adaptive or epistatic variation.

In theory, another application of molecular markers is to use them as a tool to monitor hybridization when a population is augmented with seeds from other sites. However, current research to detect hybrid individuals is reported primarily for interspecific hybrid zones and rarely addresses issues in restoration [53,54]. In the future, highly polymorphic genetic markers could be used to distinguish among introduced and local conspecific populations. This will enable the detection of genetic swamping and the identification of F1 (and possibly later) hybrids that might pose risks for the long-term sustainability of the restored population [55].

Delineation of seed zones

Seed transfer zones were first defined in recognition of strong regional differences in life-history traits of commercially important species of conifers [56,57]. These studies have compelled restoration ecologists to adopt the concept of seed zones (with new emphasis on the potential consequences of hybridization) in efforts to maximize the viability of genotypes introduced in restoration. Several investigators have reviewed the practical considerations of seed and vegetative collections that will affect restoration success [57–60]. Their recommendations, along with the methods of predicting fitness consequences of translocation discussed here, are powerful steps towards determining appropriate seed transfer zones.

First, there is growing recognition that seed collections should be made near the restoration site to ensure the genetic similarity of introduced and local populations, minimizing the probability of outbreeding depression. Recent population structure and transplant studies suggest, however, that the distance between populations is not always the best indicator of population genetic similarity [9,10,48,50]. Instead, genetic divergence among populations is likely to reflect spatial environmental heterogeneity [40]. As a result, careful efforts should be made to match germplasm for both abiotic and biotic factors, such as elevation, soil characteristics, climatic regime, pathogens and predators.

Second, it is important that seed transfer zones incorporate life-history characteristics of the focal species. In particular, the mating system of plant taxa will determine patterns of gene flow and levels of within and among population differentiation. [61]. Highly outcrossing taxa are less likely to represent ecotypic or epitypic differentiation because of the homogenizing effect of gene flow. By contrast, highly inbreeding taxa are likely to form ecotypes and/or epitypes because of their greater isolation and independent evolution. Seed transfer zones will therefore be larger for outcrossing species given that populations linked by long-distance pollen dispersal are less likely to exhibit outbreeding depression if they hybridize.

As a final consideration, once a seed zone is delineated, collections must be made from a large enough number of individuals to represent population variation adequately and to avoid severe genetic bottlenecks. Combining these principles with empirical studies of ecotypic and epitypic differentiation, gene flow and fitness in both F1 and F2 (and later) hybrids is the most promising approach to identifying the populations among which plants may be safely transferred.

Conclusions

Translocations of native species in restoration represent ‘experiments in progress’ and will open new avenues of research in the study of ecotypic and epitypic variation, and intraspecific hybridization (Box 3). It is clear that founder events and inbreeding depression should be avoided. However, there are gaps in our knowledge about the magnitude of outbreeding depression in augmented populations and its consequences for population persistence. Indeed, it is unknown whether natural selection will eliminate poorly adapted genotypes generated by translocation, reducing the need to consider outbreeding depression in restoration. Ultimately, strong research programs must be combined with monitoring by land managers to develop restoration protocols not only for the reconstruction of threatened populations, but also for the maintenance of their evolutionary potential in the face of future environmental change.

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