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Genetic Considerations in the Restoration of Small Forest Populations: Perspectives From Fish and Wildlife Genetics

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The evolutionary forces that drive recent population genetic changes include migration, mating strategy, genetic drift, and selection. However, the strength of these forces varies depending on population size. The purpose of this article is to review genetic issues associated with small forest populations and to provide perspectives from fish and wildlife genetics through case studies. Small populations are often fragmented, potentially preventing migration. In forest trees, long-distance pollen dispersal and highly mobile, generalist pollinators can help maintain connectivity. A landscape and community approach to understanding connectivity is critical (case study: mussels). Outbreeding depression can also be a concern in forest restoration. This becomes a greater risk when mixing populations that are highly diverged and when the species is polyploid. Management units should be designated that mimic natural gene flow (case study: lake sturgeon). At the other extreme, inbreeding depression can result in reduced fitness. When inbreeding depression is a concern, genetic rescue may be necessary (case study: Florida panther). Loss of diversity through genetic drift can occur with small effective population sizes ($N_e$) and a small number of founders (case study: salmonids). Selection is most likely to occur through adaptation to captivity or introduction of resistant/tolerant strains (case study: amphibians).
INTRODUCTION

Many forest populations that are a focus of ecosystem restoration efforts have a reduced number of individuals relative to their historic population sizes. These populations have often been impacted either directly or indirectly by human activities—such as unsustainable harvest, disease, and introduced species. Small population size can result in low genetic diversity, reducing the ability of populations to adapt to changing environments and further increasing the probability of extinction. The strength of evolutionary forces that result in genetic change varies, depending on population size; therefore, small and large populations may need to be managed differently in order to protect small populations from a further loss of genetic diversity.

Evolutionary forces that result in a genetic change in populations include mutation, migration, genetic drift, mating strategy, and selection. Mutation is a relatively slow process, with the average mutation rate being one new mutation per locus per 100,000 gametes per generation (Frankham, Ballou, & Briscoe, 2010). The focus of this article is on drivers of recent evolutionary change and the influence of mutation on genetic change in populations will not be considered. Migration can result in an increase in genetic diversity (due to immigrants carrying new genetic variants) or a decrease in genetic diversity (due to emigration without subsequent immigration; Frankham et al., 2010). Small populations are often small due to habitat fragmentation and are isolated from other populations. Lack of migration can then result in a loss of genetic diversity in the population. Genetic drift involves random changes in allele frequencies due solely to chance and always results in a loss of genetic diversity. Genetic drift is more pronounced in small populations and becomes the primary evolutionary force driving genetic change in small populations. Selection can either maintain genetic diversity (e.g., heterozygote advantage) or decrease genetic diversity (e.g., directional selection). In small populations, genetic drift can overpower the effects of selection (Kimura, 1983). However, selection is an important consideration when creating restoration plans and developing restoration goals. An extensive body of literature exists which examines these population genetic concepts in more detail as they relate to forest genetics (Wright, 1976; Adams, Strauss, Copes, & Griffin, 1992; Baradat, Adams, & Mueller-Starck, 1995; Young, Boshier, & Boyle, 2000; White, Adams, & Neale, 2007), as well as the issues faced by small populations (Falk & Holsinger, 1991).

When restoring small forest populations, genetic management should have two goals, taking into account two different timescales. In the short term, genetic diversity should be maximized by avoiding inbreeding between
individuals in the population. In the long term, the population should have sufficient genetic diversity to maintain its evolutionary potential. In order to achieve both of these goals, high genetic diversity in restored populations should be a priority. The key consideration is changes in adaptive genetic diversity. Increased genetic diversity is not always adaptive and, conversely, low levels of genetic diversity do not always lead to reduced fitness. Genetic diversity is directly related to population size, with small populations losing genetic diversity at a faster rate. This led to the development of what was known as the “50/500 rule,” which recommended target effective population sizes ($N_e$) for avoiding inbreeding ($N_e = 50$) and maintaining long-term evolutionary potential ($N_e = 500$; Franklin, 1980). These targets are no longer used in restoration genetics and defining target population sizes is not as easy as a single target number (Jamieson & Allendorf, 2012). However, a clear link between population size and genetic diversity remains. Extensive literature addresses the application of population genetic concepts to forest restoration in greater detail (Millar & Libby, 1989; Falk, Richards, Montalvo, & Knapp, 2006).

The importance of conserving forest genetic resources (FGR) has been recognized as a critical component of sustainable forestry (Ledig, 1986; Boyle et al., 2002). For example, conservation units in Europe have been delineated in an effort to maintain the adaptive potential of targeted tree species (Koskela et al., 2013). Coverage of these conservation units focused more on maintaining genetic diversity in large, economically valuable populations (Lefèvre et al., 2013). Selection of the target species has historically been based on either its utilitarian purpose or its potential genetic value, with large populations being primarily managed by a utilitarian approach and rare populations by an ecological approach (Geburek & Konrad, 2008). Small populations face a different set of genetic challenges relative to their more abundant counterparts and therefore require different management strategies. These rare and threatened populations are an important component to successful ecosystem restoration because they contribute to overall forest biodiversity and could increase the diversity of forest production in the future (Lefèvre et al., 2013).

Lessons can be learned from the genetic management of populations from other taxonomic groups. Unfortunately, there appears to be little exchange between forest geneticists and conservation geneticists focused on wildlife and fish populations. Small populations are found in all taxonomic groups and a broader perspective would likely benefit restoration efforts for rare species. This approach could also facilitate a community genetic perspective on ecosystem restoration, with high genetic diversity in tree populations potentially having a positive impact on the genetic diversity of dependent fish and wildlife populations. The objective of this article is to provide case studies from fish and wildlife genetics to evaluate the genetic management of small forest populations. Each evolutionary force
that has the potential to shape genetic diversity in small populations will be evaluated by first reviewing what recent research has been conducted on forest populations and then by providing a perspective from fish or wildlife genetics through a case study. Fish and wildlife species were selected based on a shared life-history or genomic trait, as these can play a significant role in shaping genetic diversity (Hamrick, Godt, & Sherman-Broyles, 1992).

MIGRATION: FRAGMENTATION

Gene flow is an important mechanism for maintaining and increasing genetic diversity in populations. From a genetic perspective, migration needs to include reproduction at the site to which the individual migrated. A migrant that brings in new alleles to the population will increase that population’s genetic diversity. However, most endangered or threatened populations have small populations due to habitat loss or degradation. Changes in habitat can affect the connectivity between populations and potentially result in isolated populations, making it difficult for these populations to maintain their genetic diversity.

For sessile organisms, gene flow is mediated by environmental variables or other organisms. In the case of forest trees, the primary mechanisms of gene flow are seed dispersal and pollination. Pollen has the potential of long-distance dispersal, potentially facilitating the maintenance of genetic connectivity despite apparent habitat fragmentation. For example, in the tropical timber tree *Dysoxylum malabaricum*, pollen dispersal maintained high genetic connectivity in fragmented populations, especially in high-density stands (Ismail et al., 2012). Similar patterns were observed in other tree species (Xu, Tremblay, Bergeron, Paul, & Chen, 2012; Manoel et al., 2012). Extensive pollen flow has also been observed to compensate for blocked seed dispersal due to fragmentation (Wang, Compton, Shi, & Chen, 2012). High genetic diversity has been observed in pollen clouds (Ozawa, Watanabe, Uchiyama, Saito, & Ide, 2012). However, these results cannot be generalized across all species and situations (Aparicio, Hampe, Fernandez-Carrillo, & Albaladejo, 2012).

Effects of fragmentation may vary depending on life stage and method of pollen dispersal. In recently fragmented populations, higher genetic differentiation has been observed in juveniles compared to adults (Finger et al., 2012). Negative genetic consequences of fragmentation may not be immediately detected (Rusterholz & Baur, 2010). When wind dispersal of pollen is the primary method of gene flow, the topography could affect how well connectivity is maintained (Quinteros-Casaverde, Flores-Negron, & Williams, 2012). When a tree species is pollinated by insects, high genetic connectivity may be maintained if the insect is a generalist (Wang, Kang, Gao, & Huang, 2010) and a good disperser (Kettle et al., 2011). Some insect pollinators have
been observed to disperse beyond fragmented areas, and have used single trees and small patches as stepping stones to larger areas (Lander, Boshier, & Harris, 2010). However, if the insect pollinator cannot tolerate the conditions of the habitat matrix surrounding the fragment, gene flow can be limited (Cuartas-Hernández, Núñez-Farfán, & Smouse, 2010). Effects of fragmentation on the pollinator need to be considered when evaluating potential effects on gene flow (Steffan-Dewenter & Tscharntke, 1999).

Case Study: Mussels

Like forest trees, mussels are sessile organisms that rely on environmental variables (i.e., water flow) and other organisms (i.e., fish hosts) for gene flow. Freshwater mussels (unionids) broadcast sperm into the water, which are then passively taken up by females (Bishop & Pemberton, 2006). The larval stage (i.e., glochidia) then disperses by passively drifting downstream (Schwalb, Garvie, & Ackerman, 2010) and by attaching to fish hosts (Haag, Butler, & Hartfield, 1995). This is similar to pollen dispersal mechanisms in trees, which rely on wind and pollinators for gene flow between populations.

As described for forest trees, the potential exists for long-distance gene flow, changing the perspective on how the effects of fragmentation should be evaluated. Connectivity was observed among Lampsilis cardium populations within a watershed due to the long-distance transport of spermatozoa (Ferguson, Blum, Raymer, Eackles, & Krane, 2013). The amount of connectivity was found to be independent of population density. Transport of the spermatozoa appeared to be facilitated by passive drift. Therefore, maintenance of flow conditions may be more critical to maintaining gene flow between populations than achieving a threshold population density (Strayer et al., 2004).

Sessile organisms require a different gene flow paradigm. The reliance on environmental variables and other organisms for gene flow necessitates a landscape and community genetic perspective. Coastal topography was identified as the primary determinant of population structure in marine mussels (Nicastro, Zardi, McQuaid, Teske, & Barker, 2008). Models incorporating the physical circulation of water along the coast and in bays were able to accurately predict larval dispersal. With the increased availability of topographic and environmental data, a landscape genetic approach is becoming more feasible and models can be developed to more accurately assess connectivity between populations.

Community genetics includes evaluation of the population structure and genetic diversity of the fish host (or pollinator, in the case of forest trees). The distribution of freshwater mussel species was shown to be primarily determined by the distribution of the host fish species (Schwalb, Morris, Mandrak, & Cottenie, 2013). Genetic diversity and population structure of small, fragmented populations of Epioblasma triquetra were compared to
the genetics of their host fish *Percina caprodes*. A correlation in population structure was observed between the mussel and its host (Zanatta & Wilson, 2011). However, the correlation was not perfect, which may be a result of the geographic scale of the study. Thompson (1994) proposed the geographic mosaic concept of coevolution, where coevolution is often observed at a geographic scale greater than the population level but below the species level. In the case of *E. triquetra-P. caprodes*, genetic diversity was not correlated. However, in another mussel-host fish relationship (*M. margaritifera-S. trutta*), genetic diversity was significantly correlated (Geist & Kuehn, 2008).

Differences in whether genetic diversity is correlated between mussels and their fish host may be attributable to differences in life history and community characteristics (e.g., density, species diversity, interaction with host; Zanatta & Wilson, 2011).

Spatial elements and biological traits of the organism interact to determine the factors influencing gene flow, making it difficult to generalize between species. However, models can be developed that incorporate both the physical landscape and organism biology and, when combined with genetic data about population structure, can make predictions about dispersal and the effects of fragmentation (Hughes, Schmidt, & Finn, 2009). For example, Meffe and Vrijenhoek (1988) proposed two zoogeographic models initially for desert streams and subsequently applied to freshwater streams in general. The Death Valley model applies to isolated remnant populations with small population sizes and loss of genetic diversity due to genetic drift. In this zoogeographic model, there are small remnant populations that used to be part of a large interbreeding population. Due to small population size and no migration, divergence between populations has been rapid. The stream hierarchy model applies to connected streams, with the level of connectivity and gene flow determined by spacing of populations within a hierarchical network of streams. In this model, there is some gene flow between populations based on geographic connectedness. These models also correspond to particular life history traits. For example, the stream hierarchy model is most applicable to habitat generalists (Hughes et al., 2009). Additional models have been developed that predict gene flow for habitat specialists (e.g., the headwater model; Finn, Blouin, & Lytle, 2007). Models that include both the landscape and the community can result in generalizations about how gene flow may be affected by fragmentation.

**MIGRATION: OUTBREEDING DEPRESSION**

Gene flow is generally viewed as a mechanism for increasing a population’s genetic diversity. However, when migrants breed with individuals from a genetically distinct species, subspecies, or population, outbreeding depression may result. Outbreeding depression is reduced fitness in the resulting
offspring relative to the average fitness of the two parents. It can result from a loss of necessary adaptations in the populations (with fitness consequences usually observed in the F1 generation) or from a breakdown of coadapted gene complexes (with fitness consequences observed in the F2 generation; Lynch, 1991). The fitness of the population may eventually recover after several generations through purging; however, with small populations and long-lived species, there is the risk that the short-term decrease in fitness could cause the population to go extinct before purging can occur (Hwang, Northrup, Peterson, Kim, & Edmands, 2012). When restoring ecosystems, the probability of outbreeding depression needs to be considered when selecting source populations.

Crossing individuals of different subspecies or populations can result in either heterosis (increased fitness of hybrids) or outbreeding depression. These two outcomes obviously have very different consequences for restoration so it is critical to be able to predict which outcome is more likely to occur. It is difficult to experimentally evaluate the likelihood of these outcomes on species with long generation times. However, experiments on species with short generation times generally show that outbreeding depression is more likely to occur when crossing highly diverged groups (Hwang et al., 2012; Pekkala, Knott, Kotiaho, Nissinen, & Puurtinen, 2012). Frankham et al. (2011) proposed that the probability of outbreeding depression is greater when the groups are distinct species, have fixed chromosomal differences, have had no gene flow within the last 500 yr, or use different environments. Several studies have demonstrated that an optimal outcrossing distance exists, most often corresponding to an intermediate geographic distance (Edmands, 2002; Grindeland, 2008; Hufford, Krauss, & Veneklaas, 2012; Sletvold, Grindeland, Zu, & Agren, 2012).

Another consideration when evaluating the likelihood of outbreeding depression is polyploidy, although polyploidy is not very common in forest trees. Theory has predicted that polyploids are more vulnerable to outbreeding depression due to the breakdown of coadapted gene complexes (Allendorf & Waples, 1996). Crossing individuals of different ploidy levels often results in sterile offspring and is therefore a straightforward example of outbreeding depression. However, ploidy is often unknown, and diploid and tetraploid individuals have been shown to both occur within the same seed transfer zone (Severns, Bradford, & Liston, 2013).

In forestry, outbreeding depression may be less of a concern than the other extreme: inbreeding depression (discussed in the next section). As discussed in the previous section, pollen and seeds can often travel long distances without any signs of the resulting offspring experiencing outbreeding depression. There are also many examples of successful interspecific crosses (e.g., Shasta fir *Abies procera* × *magnifica*) where successful hybrids are produced. With outbreeding, novel genetic combinations can be generated after several generations, potentially resulting in higher fitness.
This was observed in *Chamaecrista fasciculata*, where the F₆ generation had greater lifetime fitness than earlier generations (Erickson & Fenster, 2006). Heterosis can potentially compensate any decreases in fitness that may result from the disruption of coadapted gene complexes (Fenster & Galloway, 2000). In conifers, polyembryony (multiple embryos within an ovule, with only one embryo ultimately forming the seed) occurs, where selection for the most fit zygote can occur. Therefore, outbreeding depression could potentially be purged at that stage if selective differences existed at that life stage. Outcrossed seeds have been shown to outcompete selfed seeds when a high proportion of selfed embryos are present (O’Connell & Ritland, 2005), lending support to the idea that inbreeding depression may be a greater concern for trees.

In forest genetic management, seed transfer zones have been delineated in order to try to balance the negative fitness consequences of both inbreeding and outbreeding. Through the transfer of seeds between populations within the zones, genetic rescue of small inbred populations can occur. Previous focus on selecting seeds from local sources to avoid outbreeding depression could result in seeds coming from populations with low genetic diversity (Broadhurst et al., 2008). Methods for determining provenances include using local seed sources (local provenancing), seeds that have been shown experimentally to be adapted to the site (predictive provenancing), mimicking natural gene flow (composite provenancing), and mixing seeds from various locations (admixture provenancing; reviewed in Breed et al., 2013). Arguments for mixing seed sources include that, with climate change, seeds adapted to current conditions may not be adapted to future conditions (Hancock & Hughes, 2012; Breed, Stead, Ottewell, Gardner, & Lowe, 2013).

Case Study: Lake Sturgeon

Lake sturgeon *Acipenser fulvescens* are a long-lived species with late sexual maturity, making them a difficult species on which to experimentally study the probability of outbreeding depression. They are also predominantly tetraploid, with approximately 25% of their genome in the disomic state (Welsh, Blumberg, & May, 2003). Therefore, they may be at a heightened risk for outbreeding depression compared to diploid fish species.

Many lake sturgeon populations throughout the Great Lakes have experienced drastic declines. In an effort to accelerate recovery of the species in the Great Lakes, management agencies have been interested in stocking sturgeon to places where they have been extirpated and to supplement existing remnant populations. Although many existing populations are small, genetic diversity was not correlated to population size (DeHaan, Libants, Elliott, & Scribner, 2006) and there is no evidence of inbreeding depression in any of the populations. Therefore, the genetic management concern was to preserve the existing population structure and avoid the risk of outbreeding depression.
Genetic stocking units (GSUs) were delineated to identify appropriate donor populations for stocking (Welsh et al., 2010). Many different criteria have been published for the designation of evolutionarily significant units and management units (Waples, 1991; Moritz, 1994; Crandall, Bininda-Emonds, Mace, & Wayne, 2000; Fraser & Bernatchez, 2001; Palsbøll, Bérubé, & Allendorf, 2006); however, we used the term “genetic stocking units” instead of management units because these units are designed to maintain genetic diversity for a single management purpose (i.e., stocking). The units may not be applicable to other management goals and it is important to customize units relative to the particular management goal.

Genetic stocking units were defined based on the genetic similarity of spawning populations, with genetically similar spawning groups forming a single unit (Figure 1). Therefore, the units represented areas of gene flow. Sometimes, the units covered a relatively short geographic area (GSU 5); however, sometimes wide dispersal occurred and the unit included several lakes (i.e., GSU 1). Spawning populations were analyzed using neutral microsatellite loci, which are useful at tracking relatively recent gene flow. Genetic differentiation was measured using pairwise $F_{ST}$ values, a neighbor-joining tree based on genetic distance, and a Bayesian approach of clustering populations based on genetic data alone. Designation of GSUs was based on consistencies among the three different approaches. This model of GSU designation is similar to composite provenancing by selecting sources that replicate natural gene flow. The GSU designations cannot be static and may need to be altered in the future to reflect changing environmental conditions. Genetic rescue was not necessary for any of the populations; therefore,

![FIGURE 1](image-url)
the focus was selecting source populations that reflect natural gene flow to reduce the risk of outbreeding depression. We cannot be certain that outbreeding depression would occur. However, since inbreeding depression was not a concern, the goal was to prevent the other extreme.

INBREEDING

At the other end of the spectrum, mating with close relatives and selfing can be a concern because that mating strategy can also result in reduced fitness of the offspring (i.e., inbreeding depression). In small populations with limited gene flow, individuals may have few choices except to mate with a relative. Although connectivity often remains intact between fragmented tree populations, these populations tend to have higher levels of inbreeding and higher selfing rates (Aguilar, Quesada, Ashworth, Herrerias-Diego, & Lobo, 2008; Manoel et al., 2012; Xu et al., 2012), particularly in low-density stands (Ismail et al., 2012).

Inbreeding becomes a primary concern in ecosystem restoration when it results in inbreeding depression. Inbreeding does not always result in inbreeding depression, as some populations appear to tolerate high levels of homozygosity with no apparent fitness consequences (e.g., Pinus torreyana; Ledig & Conkle, 1983). When a population has reduced fitness due to inbreeding depression, then genetic rescue (or introducing individuals from another population) may be needed. When there is limited gene flow from other populations, inbreeding depression is a significant concern (Silva, Hardner, Tilyard, Pires, & Potts, 2010). Inbreeding results in increased homozygosity in the population, which can result in an unmasking of deleterious recessive alleles. This is the most common mechanism for inbreeding depression. Del Castillo, Trujillo-Argueta, Sanchez-Vargas, and Newton (2011) observed a positive correlation between fitness (seed viability, seedling performance), genetic diversity (heterozygosity), and population size in Pinus chiapensis. Interestingly, seed viability peaked at intermediate heterozygosities, suggesting a balance between inbreeding and outbreeding depression.

Inbreeding does not always result in inbreeding depression. It has been suggested that plants (especially self-compatible plants) often are more tolerant of inbreeding (Husband & Schemske, 1996). During the first few generations of inbreeding, the deleterious recessive alleles are selected against and effectively purged out of the population, increasing the fitness of subsequent generations. Again, as with outbreeding depression, caution needs to be exercised when working with small populations because the population may go extinct before the deleterious alleles can be purged. Purging has also been found to be most effective through within-family selection; when inbred individuals compete with individuals from other families (which is
likely in instances of high dispersal), selection is not as strong and the alleles may not be purged (Ferriol, Pichot, & Lefèvre, 2011).

Polyploidy may also decrease the probability of inbreeding depression in plants (Lande & Schemske, 1985; Ronfort, 1999). One of the mechanisms for inbreeding depression is unmasking of deleterious recessive alleles due to increased homozygosity. In polyploid organisms, the extra chromosomal copies delay homozygosity, which will therefore delay expression of the deleterious recessive phenotype. However, the taxon’s mating system is likely a more important predictor of inbreeding depression (Barringer & Geber, 2008).

Case Study: Florida Panther

In fish and wildlife genetics, genetic rescue is generally considered to be necessary when the population shows signs of inbreeding depression, there is a suitable donor population, and (ideally) experimental evidence exists supporting the likely success of the introduction (Hedrick & Fredrickson, 2010). In the case of the Florida panther (Puma concolor coryi), the necessity of genetic rescue was clear. This subspecies that once ranged throughout the southeastern United States was now restricted to less than 30 individuals by the 1980s in Florida (McBride, McBride, McBride, & McBride, 2008). Clear signs of inbreeding depression were visible in the population—including low sperm quality, many individuals with one or no descended testes, atrial septal defects, cowlicks, and kinked tails (Roelke, Martenson, & O’Brien, 1993; Barone et al., 1994; Mansfield & Land, 2002). In 1995, eight females from the Texas subspecies (P. c. stanleyana) were introduced into the Florida population, and five of the females successfully bred (Johnson et al., 2010).

Since the introduction of the Texas subspecies, the incidence of inbreeding traits has decreased (Mansfield & Land, 2002; Onorato et al., 2010), heterozygosity has increased (Johnson et al., 2010), and the population size has increased (McBride et al., 2008). The F1 hybrids had higher survival (Benson et al., 2011; Hostetler et al., 2010). However, both the F1 hybrids and the backcross with the Texas subspecies had lower reproduction probabilities (Hostetler et al., 2012). This could be due to outbreeding depression but is unlikely given the low levels of genetic differentiation between North American panthers and the historic gene flow between the two subspecies (Hostetler et al., 2012). Instead, the authors attribute the lower reproduction probabilities to higher survivorship of the hybrids’ offspring (panthers will not reproduce when they have dependent offspring). Despite the success of the genetic rescue of the Florida panther, concerns have been raised about swamping of the Florida panther ancestry due to a large proportion of offspring descending from the Texas females (Hedrick 2010; Maehr & Lacy, 2002).
GENETIC DRIFT

The greatest force of evolutionary change in small populations is often genetic drift, or changes in allele frequencies due to random chance. Genetic drift always results in a loss of genetic diversity, with the end result being increased homozygosity and ultimately the fixation of a single allele at a locus. The rate of fixation depends on the population size and the initial genetic diversity in the population. In small populations, genetic drift has the potential to overpower adaptive evolution (Ellstrand & Elam, 1993).

From a genetic perspective, the critical population size is the effective population size ($N_e$), not the census population size ($N_c$). The effective population size is often much less than the census size, with many wild populations having an $N_e$ that is 10% of $N_c$ (Frankham, 1995). The effective population size is the size of an ideal population that would lose genetic diversity (e.g., heterozygosity, inbreeding) at the same rate as the observed population. Characteristics of an ideal population include discrete generations, equal sex ratios, stable population size, equal family sizes, and random mating. When these characteristics are violated, the effective population size is reduced.

For restoration efforts, it is important to identify the number for introduction based on a target $N_e$. The first step involves identification of a target $N_e$ that will maximize genetic diversity in the restored population. The “50/500 rule” attempted to do this, proposing an $N_e = 50$ to minimize inbreeding in the short term and an $N_e = 500$ to maintain evolutionary potential in the long term (Franklin, 1980). However, Lynch (1996) proposed that an $N_e = 1,000$ was necessary to maintain adaptive potential in mutation-drift equilibrium. An $N_e = 5,000$ should be able to meet that target, while also capturing rare alleles (Yanchuk, 2001). However, most examples in forest restoration have a much lower target $N_e$.

Target effective population sizes reported in the literature include 100 (requiring at least 38 seed trees; Bessega, Pometti, Ewens, Saidman, & Vilardi, 2012), 150 (need at least 76 seed trees; Manoel et al., 2012), and 500 (from at least 133 seed trees; Ferreira et al., 2012). The primary causes for reduced $N_e$ in tree populations are non-random mating (i.e., inbreeding) and high variance in family size. Collection of seed trees from multiple fragments could reduce the effect of inbreeding on $N_e$ (Manoel et al., 2012). Lifetime fecundity variation (which reduces $N_e$) appears to be less in long-lived perennials compared to annuals, perhaps due to strong density-dependent mortality in long-lived plants prior to reaching the reproductive phase (Dodd & Silvertown, 2000). Equalizing parental contribution can result in a higher $N_e$, but there is a trade-off with lower seedling production. Optimal family size can be identified where both genetic diversity and seedling production are maximized (Kang & Lee, 2008).
Drift is the cause of genetic diversity loss observed during population bottlenecks and the founding of new populations. During both bottlenecks and founding events, subsequent generations are based upon a small subset of the original population, and thus a small subset of the original population’s genetic diversity. This can result in restored populations having lower genetic diversity than the donor population. A restored population of *Cyclobalanopsis myrsinaefolia* had fewer alleles than its donor population (Liu, Chen, Zhang, & Shen, 2008). Lower genetic diversity was observed in artificially managed ex situ populations of *Polemonium kiushianum* due to a small number of founders (Yokogawa, Kaneko, Takahashi, & Isagi, 2013). Simulations demonstrated that at least 1,000 more seeds would need to be introduced in order to overcome the diversity loss caused by genetic drift. The founder effect has even been observed in cases of natural regeneration (Hoban, Schlarbaum, Brosi, & Romero-Severson, 2012).

Revegetation techniques may play a role in the probability of a founder effect (Montalvo et al., 1997). With transplantation, only a small number of clones may actually be transplanted. This was observed in eelgrass beds, which had lower genetic diversity compared to their natural counterparts (Williams & Davis, 1996). However, a subsequent study on eelgrass beds found no effect of revegetation technique on genetic diversity (Lloyd, Burnett, Engelhardt, & Neel, 2012), so there are likely other variables that are affecting genetic drift. Studies on a conifer (*Araucaria nemorosa*) found revegetation technique to have an effect on genetic diversity (Kettle, Ennos, Jaffre, Gardner, & Hollingsworth, 2008). Nursery populations established from cones collected from adult trees showed signs of a genetic bottleneck. Those populations established from seeds collected from the forest floor did not experience a bottleneck but were inbred compared to the donor population.

**Case Study: Salmonids**

Fish conservation programs in the hatchery provide a good example for the potential effects of restoration on genetic drift and effective population size. As with many plant species, large numbers of offspring can be obtained from a single parent pair, making it tempting for managers to restore populations based on a small number of founders. Loss of genetic diversity from few founders has been observed in introduced salmonids in South America (Valiente, Juanes, Nuñez, & Garcia-Vazquez, 2007). However, in that case, it did not appear to impede the successful adaptation of the introduced species.

Wild populations of Pacific salmonids (i.e., salmon and trout) have experienced drastic declines and hatcheries have played an important role in restoring populations and supplementing existing remnant populations. In these captive breeding programs, a small number of individuals are often
used as the founders and there is typically high variance in reproductive success, with a small proportion of founding individuals contributing the bulk of the offspring. Therefore, the effective population size of the captive population may be much less than the wild population. Large numbers of the captive-born offspring (with the low $N_e$) are then usually released into the wild population, which can result in a lower overall $N_e$ (sometimes lower than what it would be without supplementation) and reduced genetic diversity. This is known as the Ryman-Laikre effect (Ryman & Laikre, 1991; Ryman, Jorde, & Laikre, 1995).

The Ryman-Laikre effect has been documented in hatchery supplementation of a steelhead population in the Hood River, Oregon (Christie, Marine, French, Waples, & Blouin, 2012). The program had a low effective number of breeders, resulting in lower genetic diversity, higher relatedness, and different allele frequencies in the hatchery fish relative to the wild population. Large numbers of offspring were produced in the hatchery program, doubling the number of spawners in the wild each year. However, the resulting $N_e$ was reduced by one-third relative to what it would have been without supplementation. As the reproductive success of the hatchery fish in the wild increased, $N_e$ was reduced further.

The problem presented by the Ryman-Laikre effect can be minimized by careful management of $N_e$ in the hatchery and the relative contribution of introduced individuals. If $N_e:N_c$ is greater in the hatchery population than in the wild population, then the overall $N_e$ can increase from supplementation (Christie et al., 2012). This could be done by reducing variance in family size. However, this increase would only be realized if the relative contribution of the hatchery individuals in the wild is low and contribution needs to be gauged by reproductive success (Christie et al., 2012).

**SELECTION**

Natural and artificial selection can influence population genetic diversity. Directional selection is when the most extreme phenotype (often homozygotes) has a fitness advantage, resulting in a loss of genetic diversity in the population. Stabilizing (or balancing) selection favors the intermediate phenotype (often heterozygotes) and is a mechanism for maintaining a population’s genetic diversity. Disruptive selection favors both phenotypic extremes, with selection against the intermediate phenotype. Outbreeding depression is an example of disruptive selection.

Selection has the strongest influence in large populations. Genetic drift is of primary concern in small populations. When the strength of selection falls below $1/2N_e$ (for diploid populations), then alleles no longer behave as if they are experiencing selection, but instead are more subject to genetic drift (Kimura, 1983). Inbreeding and outbreeding depression are forms of selection and are of obvious concern in small populations. Selection in small
populations is also a concern when individuals become adapted to conditions in captivity (making them less fit for life in the wild) and when endangered populations are not adapted to novel environmental conditions.

Small populations may not be able to adequately adapt to changing environments, particularly climate change. The climate has continually cycled throughout history and forests have appeared to lag behind their environmental optima, always struggling to catch up to an ever-changing climate (Westfall & Millar, 2004). Now, however, the climate is changing at a faster rate and forests are experiencing multiple threats simultaneously. In response to climate change, species can migrate to a different location, adapt to the new conditions, or be extirpated. Migration and adaptation are not mutually exclusive. Species with high fecundity, large population sizes, short generation times, and small seeds that disperse easily are the ones that will most likely survive and adapt to climate change (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008). The field of landscape genomics can provide insight into potential barriers to migration, the likelihood that a population can adapt, and the identification of locations that lack sufficient genetic diversity to adapt (Sork et al., 2013).

Gene flow can potentially have both positive and negative effects on a population’s response to climate change (Kremer et al., 2012). The positive effect can be increased genetic variance, allowing the population to adapt. The negative effect can be homogenization of populations, constraining adaptive divergence. The positive effect usually outweighs the negative, but the result can depend on region (Kremer et al., 2012). The leading edge and the core area of the range has the benefit of receiving alleles from southern populations that are adapted to the new climate, whereas the trailing edge does not get that same benefit (Hampe & Petit, 2005).

Adaptation to the changing climate is another strategy for forest trees. Genes that are likely to experience the strongest selection due to climate change are genes that regulate growth timing (Howe et al., 2003). Small populations with low levels of genetic diversity, low fecundity, and long generation times will likely be the least able to adapt (Aitken et al., 2008). Population demography also plays a role in a species’ ability to adapt to changing climate. Species with high mortality and regular disturbances (that result in mass mortality) may be able to better adapt (Kuparinen, Savolainen, & Schurr, 2010). The high mortality eliminates maladapted individuals, allowing for better adapted seedlings to be produced and established.

For species that have a low likelihood of migrating or adapting, alternative approaches need to be considered. One approach is assisted migration, where species or populations are actively moved in response to climate change to simulate natural range expansion (Peters & Darling, 1985). Decision tools have been created to evaluate a species’ risk of extirpation and its likelihood of migration (Hoegh-Guldberg et al., 2008), identifying potential candidates for assisted migration. Research efforts seek to evaluate the feasibility of assisted migration. For example, the Assisted Migration
Restoration of Small Forest Populations

Adaptation Trial is a collaborative effort between the United States and Canada, where the performance of northern populations of different tree species is tested in warmer climates (Williams & Dumroese, 2013). Seed zone boundaries will need to be re-evaluated to take into account future climatic conditions, which would likely require crossing existing seed zone boundaries (Williams & Dumroese, 2013).

Novel pathogens also create an environment in which strong selection can occur. American chestnut (Castanea dentata) populations were ravaged by chestnut blight (Cryphonectria parasitica), a pathogenic fungus introduced in the early 1900s. Introduction of hybrids and backcrosses with the blight-resistant Chinese chestnut (C. mollissima) or Japanese chestnut (C. crenata) has been a successful management strategy (Griffin, 2000). However, although selection is for a particular trait (i.e., blight resistance), other genes that may not be as fit also come along. For example, nuts of the hybrids and backcrosses are not as hardy at cold temperatures, potentially limiting restoration potential in northern latitudes and at high elevations (Saielli, Schaberg, Hawley, Halman, & Gurney, 2012).

There is a distinction between resistance and tolerance, with the implications being critical for successful conservation (Venesky, Mendelson, Sears, Stiling, & Rohr, 2012). Resistance reduces (or eliminates) the effects of the pathogen or herbivore by either preventing a response or attacking the pathogen/herbivore. This can result in a potential evolutionary response by the pathogen, resulting in increased virulence. Alternatively, tolerance permits the attack, but the reduction in fitness is minimized. This approach likely has a neutral effect on coevolution with the pathogen/herbivore. Therefore, Venesky et al. (2012) argue that selecting for host tolerance (not resistance) should be the preferred strategy to maximize the probability of long-term persistence for the population.

Case Study: Amphibians

Worldwide, populations of amphibians are experiencing drastic declines due, in part, to a lethal fungal pathogen Batrachochytrium dendrobatidis (Bd; Vredenburg, Knapp, Tunstall, & Briggs, 2010; Cheng, Rovito, Wake, & Vredenburg, 2011). A genome-wide study found that Bd had a common effect on several susceptible frog species (Rosenblum, Poorten, Settles, & Murdoch, 2012). Significant differences in the expression of genes responsible for maintaining skin integrity were observed between infected and noninfected frogs. No significant expression difference in immune-related genes was observed.

Most amphibian species and populations in the wild are not able to effectively respond to Bd in the wild; therefore, efforts are being made to use captive programs to prevent extinction. Some amphibian species and populations appear to be faring well despite the presence of Bd (e.g., Briggs,
This variation in response to Bd, particularly within populations, could be important in selecting tolerant individuals. Bd evolves faster than its host, so releasing resistant individuals may not be a good approach; instead, tolerant individuals should be selected (Venesky et al., 2012).

Both genetic and environmental factors play an important part in determining species tolerance to Bd. Variation at the major histocompatibility complex (MHC), which is involved in vertebrate immune defense, has been linked to amphibian survival in the presence of Bd. MHC heterozygotes and individuals with a particular MHC allele had greater survival when exposed to Bd (Savage & Zamudio, 2011). Populations with low genetic diversity (measured at neutral microsatellite loci) did not respond as well in the face of Bd infection as populations with higher diversity (Luquet et al., 2012). Neither group got infected, but the tadpoles from European treefrog (Hyla arborea) populations with low genetic diversity had a shorter postmetamorphic lifespan and decreased mass. Low genetic diversity put the populations at a disadvantage where they could not adequately invest in resistance, growth, and metamorphosis simultaneously. Crossing individuals of closely related species did not improve fitness in the presence of the pathogen, despite improved fitness in the absence of the pathogen (Parris, 2004). This illustrates that outbreeding depression remains a concern and that it may not become apparent until conditions change. Environmental factors can also add a complicating element to effective management for Bd tolerance. For example, amphibian immune response to Bd is temperature dependent (Ribas et al., 2009).

CONCLUSIONS

- Fragment boundaries do not necessarily equal gene flow boundaries (Kramer, Ison, Ashley, & Howe, 2008). It is necessary to incorporate a landscape and community genetic perspective to better understand what determines connectivity between forest fragments. Sessile organisms that rely on the environment and other organisms for dispersal require a different gene flow paradigm.
- One of the goals of restoration should be to balance the negative fitness consequences of inbreeding and outbreeding depression. If the population shows no signs of inbreeding depression, the risk of outbreeding depression should be minimized by designing seed transfer zones that mimic natural gene flow. If inbreeding depression (not just inbreeding) is a concern, genetic rescue may be necessary. Donor individuals should be selected from a genetically diverse population of intermediate geographic distance and from a similar environment.
• Genetic rescue may be necessary when a species or population is unable to adapt to an introduced pathogen or herbivore. If available, crosses can be made with a closely related species or population tolerant to the pathogen/herbivore. Resistant individuals should not be selected because the pathogen/herbivore is likely to adapt more quickly than long-lived plant species. It is also important to consider that more than just the gene(s) of interest are present in the hybrids. Outbreeding depression could occur if there are introduced genes that are not adapted to particular environmental conditions.

• Restoration efforts should avoid a loss of genetic diversity resulting from genetic drift. This can be done by maximizing both the effective population size and the number of founders. Family sizes should be equalized prior to introduction to the restoration site. Donors should be collected from multiple fragments to avoid the effect of inbreeding on $N_e$. If these individuals are then introduced into an existing population, their relative contribution to future generations should be monitored, if possible.

• In restoration, it is important to consider the ability of a species or population to adapt to a changing environment. Climate change and novel pathogens are likely the biggest drivers of selective change in populations. In response to these changes, a species can either migrate, adapt, or be extirpated. The likelihood of each of these options should be evaluated and management actions selected accordingly.

• It is easy to become focused on one’s particular taxonomic specialty. However, in the case of restoring genetic diversity, the greatest difference is between small and large populations, not taxonomic group. Perspectives can be gained from genetic management that has occurred in other taxonomic groups and interdisciplinary crosstalk is critical to maintaining that perspective.

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