Oregon ash (*Fraxinus latifolia* Benth. [Oleaceae]) seedling. Photo by Adrienne Basey

# Producing native plant materials for restoration: 10 rules to collect and maintain genetic diversity

**Adrienne C Basey, Jeremie B Fant, and Andrea T Kramer**



## ABSTRACT

Ecological restoration aims to assist the recovery of degraded, damaged, or destroyed ecosystems. Restoration practitioners increasingly recognize the value of using ecologically appropriate and genetically diverse native plant material to support ecosystem recovery and long-term persistence in the face of unpredictable current and future conditions. Producing genetically diverse native plant material, however, can be incredibly challenging. Each step of production, from procuring raw material to installing produced material into a restoration site, has the potential to affect the genetic diversity of the produced material. Here we examine each of the production steps, from wildland seed collection through seed or seedling production. We outline each step where genetic diversity can be lost or gained, and describe 10 rules that can be used to maintain high genetic variability in native plant material throughout the production process.

Basey AC, Fant JB, Kramer AT. 2015. Producing native plant materials for restoration: 10 rules to collect and maintain genetic diversity. Native Plants Journal 16(1):37–52.

**KEY WORDS**
native seed collection, native plant production, seed production, maintaining genetic variability

**NOMENCLATURE**
USDA NRCS (2015)

Ecological restoration, as defined by the Society for Ecological Restoration, is "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed." Now facing unpredictable current and future conditions, restoration practitioners more fully appreciate the value of using ecologically appropriate, genetically diverse native plant material in projects with the goal of long-term persistence (Harris and others 2006; Clewell and Aronson 2013). Collecting and maintaining genetic diversity when producing native plant materials for restoration, however, presents numerous challenges that require special consideration throughout the production process (Rogers 2004). Each of the steps involved, from procuring raw material to installing produced material into a restoration site, has the potential to reduce genetic diversity. In this review we outline 10 rules with the aim of helping seed and plant producers identify genetically diverse raw material and maintain that diversity throughout production.



## WHY IS GENETIC DIVERSITY IMPORTANT FOR RESTORATION?

Most plant material produced in the US and worldwide is intended for agricultural or horticultural purposes. In these cases, uniformity is a desirable trait, and management practices can be used to maintain a crop through extreme weather, fertility problems, or disease issues. Plant material produced for ecosystem restoration deviates from most cultivated plants in that it will be used to establish a population capable of persisting and reproducing under a wide variety of conditions, often without any intervention. Genetic diversity is one way that many natural populations are able to survive yearly variation in weather, disease, competition, and soil conditions; hence, using genetically diverse materials is one way of ensuring that restored populations mirror the potential of natural populations. What is important to recognize is that this genetic diversity (see glossary) may be visible only under specific situations, such as conditions of drought or a disease outbreak.

Using genetically diverse, ecologically appropriate native plant material in restoration may improve ecosystem recovery in numerous ways. For example, a number of studies have shown that, at least in some systems, the use of genetically diverse plant material can improve establishment success under a range of conditions (Crawford and Whitney 2011), increase resistance to pests and pathogens (Tooker and Frank 2012), and support faster recovery after disturbances or climatic extremes (Hughes and Stachowicz 2004; Reusch and others 2005) when compared to material with low diversity. Additionally, because genetic diversity allows populations to adapt to unpredictable and changing conditions, restored populations that are genetically diverse are more likely to adapt and persist into the future than those with limited diversity (Jump and others 2009). The benefits of genetic diversity also extend to the ecosystem functions that they provide. Research in some ecosystems has shown that genetically diverse populations are more productive, have increased nutrient retention, and support more diverse and abundant animal communities (Johnson and Agrawal 2005; Crawford and Rudgers 2012a,b; Reynolds and others 2012). This is not to say, however, that genetic diversity is a panacea, nor is it a substitute for matching the genetics of the source material to the restoration site conditions (Falk and others 2001; Withrow-Robinson and Johnson 2006). If the plant material is adapted to conditions that substantially differ from those of the restoration site, no amount of additional genetic diversity will improve the restoration's success (Johnson and others 2010).

### What Does This All Mean for Production?

Cultivated plants represent only a subset of the genetic diversity of wild populations (Barrett 1981). Under cultivation, selecting plants that possess traits considered desirable for production and performance is associated with loss of diversity. Plant production is most efficient when characteristics such as seed germination requirements, plant size and structure, or timing of flowering and seed development are uniform. Genetic diversity can translate to nonuniformity in these characteristics; hence, maintaining genetic diversity can make plant production more challenging (Kitzmiller 1990; Smith and others 2007). The lack of uniformity in genetically diverse plant material means that genetic diversity can be lost at every stage of plant production (Figure 1) and, if not considered with every step, loss or change is likely. We outline 10 genetic rules to assist in the production of restoration material that is ecologically appropriate and genetically diverse, providing justification for, and examples of why, each rule is important. These rules are intended to serve as a general road map, and not all rules will be appropriate or feasible in all circumstances; application and prioritization of each rule ultimately rests in the hands of the land manager, seed producer, or plant propagator most familiar with the species and situation. Likewise, these rules are not intended for production of threatened or endangered plant species, as specific guidance is available elsewhere (Guerrant and others 2004; Maschinski and Haskins 2012).

## PROCURING RAW MATERIAL: SITE SELECTION

**RULE 1. Identify sources with conditions similar to potential restoration sites.** Genetic diversity can vary between sites depending on local conditions. Plant material to be used in a restoration is more likely to establish and persist if it comes from a site with similar ecological conditions.

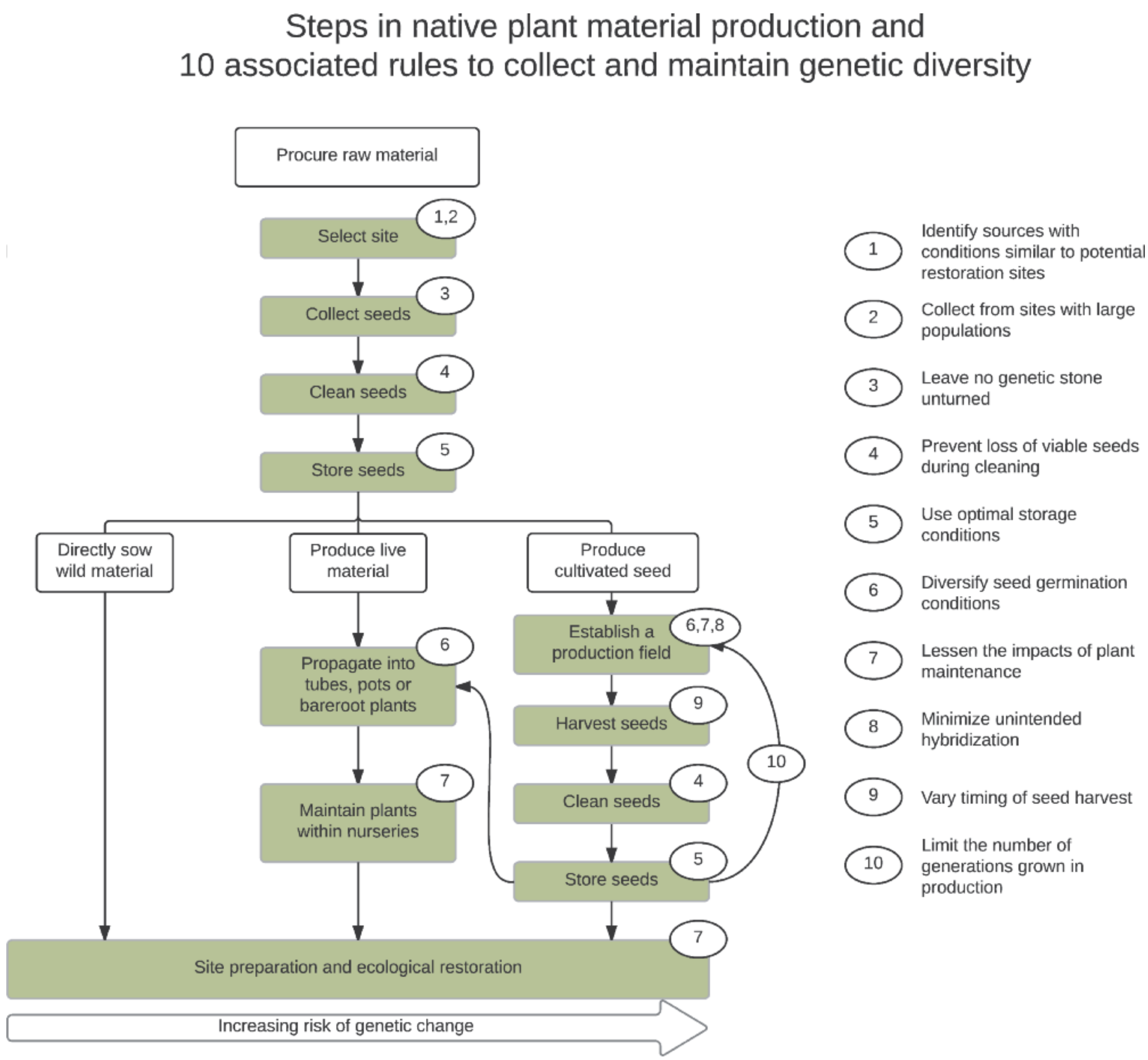


*Figure 1.* Steps in native plant material production and 10 associated rules to collect and maintain genetic diversity. Seeds or cuttings are first collected from a natural population. This material may be used to either sow wild-collected material directly into a restoration site, produce live material such as seedlings or cuttings, or produce cultivated seeds in production beds or seed orchards. Each action represented by a shaded block has inherent potential to alter genetic diversity, and these actions compound upon each other as plant material is held in production.



## Background

Decades of research have shown that plant populations are often adapted to the environmental and biological conditions where they grow (for example, soil type, rainfall, temperature extremes, pests, pathogens, and soil mycorrhizae) (Linhart and Grant 1996; Hufford and Mazer 2003). This adaptation to different local conditions represents unique and potentially useful genetic diversity. Failing to consider adaptation when sourcing native plant material for restoration may lead to poor outcomes, for example, plant material that is not adapted to site conditions may fail to germinate, grow, or reproduce, or it may do so at a lower rate than for material adapted to site conditions (Johnson and others 2004; Ågren and Schemske 2012; Bennington and others 2012; Gibbs and others 2012). Consequently, it is beneficial to target seed collection sites strategically, with some consideration of where and how the seeds will be used, rather than just selecting a site because it is accessible or convenient. While the intended restoration site(s) will be unknown for some collected or produced plant material, ensuring that detailed notes on species identification, source location, and site conditions are available will increase its value and allow end-users to match available material to restoration site.

Extensive reviews have been published during the last 2 decades to help restoration practitioners determine when adaptation may be important and how it can be incorporated into sourcing appropriately adapted material (Knapp and Rice 1994; Lesica and Allendorf 1999; McKay and others 2005; Vander Mijnsbrugge and others 2010). Recent reviews on this subject have focused on the specific issues of adaptation and sourcing for restoration in an increasingly changing climate (Broadhurst and others 2008; Breed and others 2013; Herman and others 2014; Havens and others 2015). Common themes in all of these reviews are that 1) patterns of genetic diversity and adaptation vary by species, and 2) strategies of how genetic diversity and adaptation should be incorporated in sourcing plant materials for restoration will vary depending on the agency or organization carrying out the restoration, the goals of the restoration, and the site and species being restored.

When information is limited on best practices for sourcing ecologically appropriate material for a species, land managers may conservatively seek the nearest source available (Saari and Glisson 2012) or try to source material from sites that are ecologically similar to the restoration site (MacKay 1993; Ward and others 2008; Johnson and others 2010). Seed transfer zones have been developed for a growing number of species to delineate geographic boundaries within which seeds can be moved with minimal risk of being poorly adapted, for example, Indian ricegrass (*Achnatherum hymenoides* (Roem. & Schult.) Barkworth [Poaceae]; Johnson and others 2012), broadleaf lupine (*Lupinus latifolius* Lindl. ex J. Agardh ssp. *latifolius* [Fabaceae]; Doede 2005), sagebrush (*Artemisia* spp. [Asteraceae]; Mahalovich and McArthur 2004), and most commercial tree species (Johnson and others 2004). These geographic boundaries can be used to guide seed collection, production, and restoration sourcing decisions on a species-by-species basis (Johnson and others 2004). For many species, however, the genetics research necessary to develop seed transfer zones has not been carried out. For these species, the USDA Forest Service's provisional seed transfer zones can help determine where important genetic diversity may exist (Youtie and others 2012; Bower and others 2014; Kramer and others 2015), and may be particularly useful when sourcing material to restore relatively high-quality habitat.

Alternatively, the goal of some restorations may be rehabilitating degraded or ecologically extreme habitats. In these situations, a number of other environmental considerations that are not included on any seed transfer zone map may be important for restorations. Plants growing in "ugly" sites (see Example 1), sites with extreme soils (saline, heavy metals, sandy condition), or during years with poor growing conditions may harbor unique genetic diversity that will be especially useful when restoring degraded sites (Conesa and others 2007; Leger 2008; Havens and others 2015). For example, "ugly" sites may produce plants with genetic diversity that makes them more resistant to grazing (Fahnestock and Detling 2000) or more competitive with invasive species (see Example 1). Furthermore, any plants able to produce seeds during a drought year may represent important, drought-resistant genetic diversity that will be particularly beneficial at sites increasingly exposed to drought conditions. While these sites or years are often avoided when collecting seeds, the unique genetic diversity they may hold makes them worth considering.

### EXAMPLE 1. WHEN "UGLY" SITES ARE USEFUL

Research on alkalai sacaton grass (*Sporobolus airoides* (Torr.) Torr. [Poaceae]) (Figure 2) growing in "ugly" habitat invaded by Russian knapweed (*Acroptilon repens* (L.) DC. [Asteraceae]) has shown that native plants growing in these sites may contain exactly the kind of genetic diversity needed for restoring similar degraded sites. Plants of *S. airoides* collected from "ugly" sites were more competitive with Russian knapweed and other invasive species than plants of the same species collected from undisturbed sites (Ferrero-Serrano and others 2011).

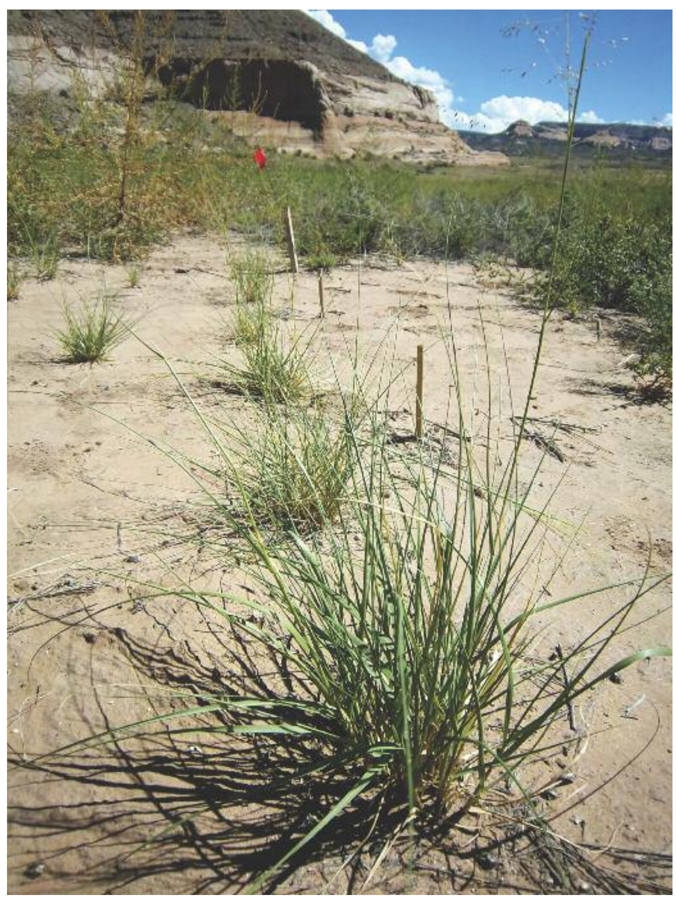

*Figure 2.* Alkalai sacaton grass (*Sporobolus airoides*) growing in an "ugly" site. Photo by Nora Talkington

### Application

1. Collect from sites with environmental conditions similar to potential restoration sites (for example, habitat, soil, climate, or presence of invasive species, drought, or disturbance).
2. Keep detailed location information for each collection, and be sure the final product can be traced back to this information.
3. Make use of seed transfer zones or similar maps that may indicate sites with useful genetic diversity for specific restoration projects.
4. Consider collecting from "ugly" sites, extreme conditions, or sites experiencing a year with poor growing conditions for specialized collections that may do well in similar circumstances.
5. Avoid sites where the target species has been included in previous seeding or planting work unless you are confident that genetic diversity is not reduced and (or) poorly adapted to the site.

**RULE 2. Collect from sites with large populations.** They generally have more genetic diversity than small populations.

### Background

Populations with more individuals often have greater genetic diversity, as supported by both theoretical and on-the-ground work (Figure 3) (Leimu and others 2006; Frankham and others 2010; Fant and others 2014). Genetic diversity increases with population size because larger populations can hold more potential diversity and because small populations tend to lose genetic diversity over time due to random events (a process called *genetic drift*). For example, consider a population with 30 plants where 3 plants (10%) have unique genetic diversity that makes them resistant to a pathogen. If a rock slide removes half of the population, the likelihood that these 3 plants will be randomly killed is higher, and with them the diversity they hold will be lost. If, however, the population had been larger, more plants would likely have maintained this unique genetic diversity, as at least some of them would have survived the rock slide. But how can you tell when a population is large enough to have experienced minimal loss of genetic diversity? The answer varies by species and situation but, in general, populations with less than 100 reproductive individuals are likely to show the negative effects of genetic drift (Montalvo and others 1997), while increasingly larger populations (for example, >1000 plants) are less likely to experience drift and more likely to maintain adaptive genetic diversity (Leimu and Fischer 2008).

Large populations will not only be likely to contain greater genetic diversity but also they will be easier to collect sufficient quantities of seeds from without negatively affecting the health of the population itself. In general, removing too many seeds from any population over multiple years will put it at higher risk of extinction (Menges and others 2004). While each species and population is different, the impacts of seed collection will be worse for populations that are exposed to other threats such as drought, grazing, pests, or competition with invasive species. Because it is difficult to tell whether a specific species or population will be negatively affected by seed collection, the Bureau of Land Management (BLM) and others recommend that no more than 20% of available seeds be collected in any given year (USDI BLM 2012), and even this amount should not be repeated at a site over multiple years.

### Application

1. Once potential priority populations have been identified (Rule 1), preferentially select sites with the largest number of individuals.
2. Select sites with sufficient plants and seeds so that no more than 20% of available seeds will be collected.
3. Prevent overharvesting and minimize impacts to native populations by working with land managers and securing appropriate permits.

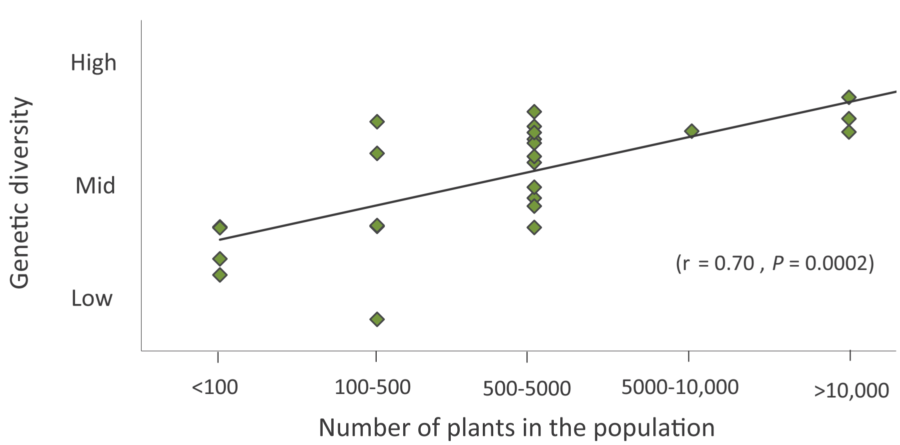


*Figure 3.* Neutral genetic diversity generally increases with population size in a native thistle, *Cirsium pitcheri* (Torr. ex Eaton) Torr. & A. Gray (Asteraceae) (modified from Fant and others 2014). The pattern for non-neutral traits will depend on selective pressures, but smaller populations are more likely than large populations to lose both neutral and non-neutral diversity through genetic drift, as seen in Figure 8.

**RULE 3. Leave no genetic stone unturned.** Collect material strategically to ensure it best represents the full genetic makeup of a population.

## Background

When collecting seeds from wild populations, the natural tendency is to collect from areas that are easy to access and that contain a high density of robust and vigorously fruiting individuals. Avoiding plants that are difficult to access or that look less robust, however, may leave an important subset of the population's genetic diversity out of the collection. Individual populations can harbor a surprising amount of genetic diversity, and seed collections should aim to capture as much of this diversity as possible. Collection techniques can affect the genetic diversity of a seed collection and, ultimately, what is available for restoration projects. While it is challenging to collect all possible genetic variation in a large population, a number of strategies can be used to maximize genetic diversity with minimal effort.

First, consider the number of individuals from which to collect seeds. The total genetic diversity captured in a collection will increase with every new, unrelated, individual sampled. It is possible to capture most of the genetic diversity found within a population by collecting from a relatively small number of unrelated plants. For example, when seeds are produced through cross-pollination, collecting from 30 *entirely unrelated* plants will capture around 95% of all but the rarest forms of diversity in the population, while collecting 45 individuals will increase that percentage to 99% (Figure 4) (Lawrence and others 1995; Crossa and Vencovsky 2011; Hale and others 2012; Hoban and Schlarbaum 2014). The only life history or ecological trait of a plant species that has been shown to influence this pattern is the type of mating system (whether a plant is purely outcrossing or is also capable of selfing) (Duminil and others 2007). For seeds that are produced through self-pollination, twice as many unrelated individuals must be collected to capture the same amount of genetic diversity (that is, 60 individuals for 95%, 90 for 99%). Unfortunately, the reproductive biology (for example, whether it reproduces by way of cross-pollination or self-pollination) of most native species is not well known, and rates of selfing can vary greatly among species, populations, and even between flowers on the same plant (Vogler and Kalisz 2001; Karron and others 2009). Because of this, the BLM and other plant specialists recommend collecting from more individuals than the absolute minimum of 30 plants to ensure that you are capturing the desired genetic diversity. For this reason, the BLM Seeds of Success program recommends a *minimum* of 50 individuals in a collection (USDI BLM 2012).

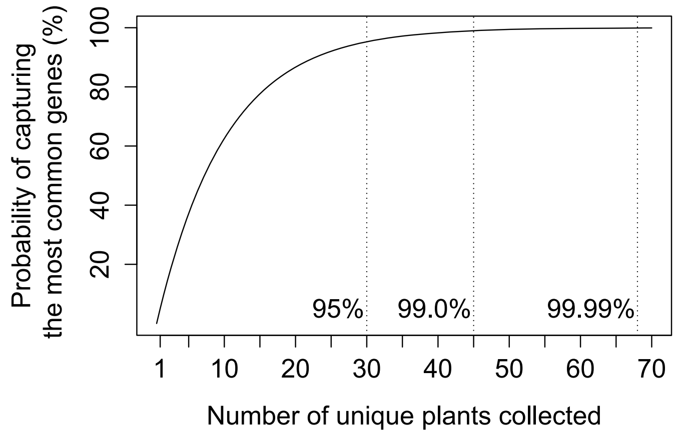


*Figure 4.* The likelihood of capturing all but the rarest forms of genetic diversity in a population increases with the number of plants collected. Dotted lines show probability of collecting the most common genes when 30, 45, and 68 unrelated, outcrossing individuals have been collected. This figure is based on probability models for cross-pollinated seeds described in Lawrence and others (1995), Crossa and Vencovsky (2011), and Hale and others (2012).

Second, minimize the collection of related individuals. Identifying which plants in a population are likely to be related can be difficult without expensive genetic analyses, but there are ways to minimize the collection of related individuals. In general, avoid collecting plants growing very close to each other to minimize the risk of collecting siblings or even clones of the same plant (Vekemans and Hardy 2004; Rhodes and others 2014)). Try to collect from plants growing throughout the whole site to ensure that you capture the full diversity present. In particular, plants growing on the edges of the population or in different microhabitats (wetter, drier, steeper) within a site may contain unique and valuable genetic diversity. Finally, do not avoid plants that look different or have unique growth forms, as these plants may hold unique genetic diversity that could be important for restoration. For example, small plants may have higher survival rates than large plants under stressful conditions (Rowe and Leger 2011).

Finally, collect seeds that are mature and healthy because those factors can have an impact on seed germination and longevity (Love and others 2014). In general, collecting seeds when natural dispersal is occurring will help ensure that seeds are mature (Baskin and Baskin 2001), and performing cut tests prior to collecting will assess seed health by identifying empty, abnormal, or infested seeds (Luna and Wilkinson 2009). Be aware that, in many natural populations, plants will not flower and produce mature seeds at exactly the same time; these differences may reflect useful genetic variation (Corre 2005). Collecting seeds when the population is at peak seed maturity and, when possible, collecting on multiple dates to ensure that early- or late-flowering plants are represented will maximize potential genetic variation in the collection. Finally, aim to collect a roughly similar volume of seeds from each plant so as to not skew the representation of one plant over others in the collection.

### Application

1. Strategically collect mature, healthy seeds from at least 50 unique, unrelated plants to maximize genetic diversity captured:
   a. Do not collect from plants growing next to each other (they are more likely to be related).
   b. Do not avoid plants that look different.
   c. Collect multiple times to capture early- or late-flowering plants.
   d. Collect from plants in all microhabitats at a site.
   e. Collect roughly similar numbers of seeds from each individual.
2. If collecting from multiple populations of the same species, use the same collecting strategy for all populations.

***Once a seed collection with maximum genetic diversity has been made following Rules 1 to 3, following Rules 4 to 10 will help ensure that this genetic diversity is not lost during the production process.***

## SEED CLEANING AND STORAGE

**RULE 4. Prevent loss of viable seeds during cleaning.** Unintentionally or intentionally removing viable seeds during cleaning may lead to loss of important genetic diversity.

### Background

Seed size is controlled by both genetic factors (Upadhyaya and others 2006) and external factors such as temperature, nutrient levels, and moisture. Such complexity means that considerable variation in seed size often exists within a population (Thompson 1981; Baskin and Baskin 2001; Obeso and others 2011). This variation can determine establishment success, predation risk, dispersal distance, and even duration of seed dormancy (Hare 1980; Rees 1996; Dalling and Hubbell 2002). Variable seed sizes can therefore represent important genetic diversity that will be useful in restoration. For example, plants germinated from smaller seeds have been shown to be more successful at establishing and persisting in stressful restoration sites (Kulpa and Leger 2013). Often when seeds are cleaned, however, the preference is to select the largest and healthiest seeds and to discard the smaller seeds. While culling nonviable seeds during the seed-cleaning process is an important aspect of propagation, it is essential that cleaning techniques do not also eliminate viable seeds of non-standard size because this can reduce valuable genetic diversity (see Example 2).

Different populations of the same species can also produce seeds with a different range of seed sizes. This means that an appropriate seed-cleaning protocol for one population may not work for another. Cleaning protocols or tools that cull seeds based on size or weight may be inadvertently selecting for larger seeds in one population but not another. For this reason it is important to clean each population separately and to alter protocols as needed. It goes without saying that it is also important to ensure that equipment is thoroughly cleaned between usage to prevent contamination with seeds from other species or even from different populations of the same species.

### Application

1. Use cleaning equipment or settings that allow the greatest proportion of viable seeds to be processed.
2. Observe chaff after cleaning to assess if smaller viable seeds have been unintentionally culled. If they have, find ways to retrieve small viable seeds from chaff.
3. Clean seeds from different seedlots separately, and modify cleaning protocols for each species and collection year as necessary.

### EXAMPLE 2. SEED SIZE AND GENETIC DIVERSITY

In Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco [Pinaceae]) (Figure 5), seed size can vary considerably between plants and years even within the same population (Silen and Osterhaus 1979). When cleaning seeds of species such as Douglas-fir, the lightest seeds may be culled out, with the expectation that large seeds are more likely to produce large, vigorous nursery plants. This is not always true and often leads to loss of genetic diversity. For example, after culling the lightest 1/3 of seeds from a single Douglas-fir seedlot (in which seeds from 18 trees were bulked), 90% of all seeds from 3 trees was lost, and more than 50% of seeds from an additional 3 trees was lost. This means that, out of 18 genetically different trees in the original seedlot, only 12 were well represented after cleaning. It is noteworthy that the 6 trees that had most of their seeds removed also produced some of the tallest saplings. This is a significant loss of potentially important genetic diversity that can be avoided if viable seeds are not intentionally or unintentionally culled.

*Figure 5.* Douglas-fir (*Pseudotsuga menziesii*) seeds showing a range of size among viable seeds. Photo by Sheree Pickens



**RULE 5. Use optimal storage conditions.** Seeds with different genetic backgrounds may vary in storage potential, with loss of seed viability over time translating to loss of genetic diversity

### Background

Once seeds are cleaned, how they are stored, and for how long, varies greatly depending on intended future use and available facilities. Seeds from different populations, and even various individuals within a population, will have varying responses to storage conditions. Some may retain their viability for a decade or more over a range of conditions, while others may lose viability rapidly (Walters and others 2005). This variability means that the death of seeds during storage can lead to a loss of genetic diversity. To prevent such a loss, maximize seed survival by using optimal storage conditions. Most angiosperms (an estimated 75–80% globally) (Walters and others 2013) produce seeds that are orthodox, meaning they can be stored for at least a year under cold, dry conditions without major losses in viability. In general, the drier and cooler the storage conditions, the longer orthodox seeds will be able to survive. Large fluctuations in humidity and (or) temperature should also be avoided.

A number of species produce seeds that are not able to survive dry storage (often referred to as *recalcitrant* rather than *orthodox* seeds), including oaks (*Quercus* L. species [Fagaceae]) and buckeyes (*Aesculus* L. species [Hippocastanaceae]). These species generally cannot be stored for long durations under any conditions (except under extremely cold conditions using cryopreservation; see Walters and others 2013 for examples). Storage in cool moist conditions for a short time can, however, help maintain as much viability as possible (see Bonner 2003 for specific recommendations on the collection and care of oak acorns). Regardless of whether seeds are orthodox or recalcitrant, storage recommendations can vary depending on the species; general guidelines have been described in a number of publications (Guerrant and others 2004; Bonner and Karrfalt 2008; Luna and Wilkinson 2009).

### Application

1. Store seeds under optimal conditions; these will vary by species, but in general, for species with orthodox seeds:
   a. Ensure seeds are dry and have low moisture content (when feasible, store seeds in an airtight container with silica gel packets).
   b. Ideally, store seeds in cold conditions (at, or slightly above, freezing) at low relative humidity.
   c. If seeds are stored at room temperature, relative humidity must be very low (works best in arid regions). In general, the rule of thumb that the storage temperature (°F) plus the relative humidity (RH%) should be less than or equal to 100 can be effective for orthodox seeds stored for a year or less.
2. For species with recalcitrant seeds, store in moist, cool conditions and use shortly after harvesting.
3. Do not store seeds longer than necessary, especially under suboptimal conditions.
4. Store seeds from different populations separately in order to track and manage losses in viability.

## PLANT PRODUCTION CONSIDERATIONS

**RULE 6. Diversify seed germination conditions.** Seeds that do not germinate may represent a loss of genetic diversity.

### Background

When seeds are grown out for production, the preference is often to select the first plants to germinate and the largest seedlings for production until the quota of plants is filled. Seed germination in natural populations, however, is rarely synchronous; separate populations, and even individuals within a population, may differ in seed germination requirements. Consequently, the initial flush of seedlings in a bulked collection may represent only a small subset of individuals collected (Figure 6). A nursery, seed orchard, or production field that utilizes only this first flush may have immediate genetic losses or unwanted shifts in genetic diversity (Knapp and Rice 1994; Cabin and others 1997; Ensslin and others 2011). To minimize this loss, expose seeds to a range of germination conditions, and make sure as many seeds germinate as possible. Also, because early-germinating seedlings have longer to grow, they are often larger and therefore more likely to be preferentially outplanted

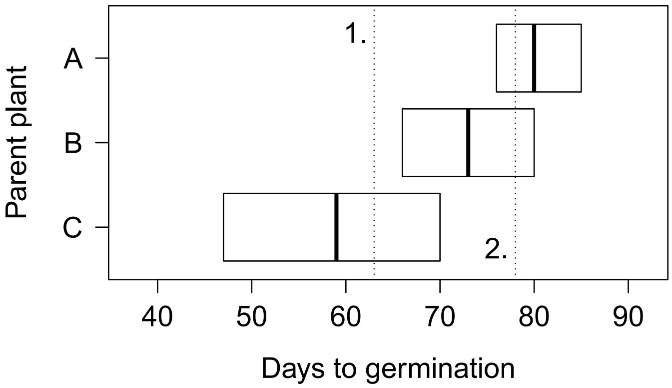


*Figure 6.* Range of germination timing for seeds collected at the same time from 3 individual *Penstemon pachyphyllus* A. Gray ex Rydb. (Scrophulariaceae) plants (A, B, C) growing in the same population, demonstrating the potential for loss of genetic diversity. The left edge, right edge and center line of each box represents the first, last, and average flush of germinating seedlings, respectively. Selecting seedlings from the first cull (Line 1. at 62 days) would represent only 1 individual, while selection during the second cull (Line 2. at 78 days) would represent 2 individuals. At no time is there simultaneous germination of all 3 individuals (Kramer unpublished data).



over smaller late-germinating seedlings, further driving loss of diversity. It is therefore important to ensure that all seedlings survive through production, and that plants that germinate first do not make up the entire production line.

Why is this important in a restoration? Germination requirements are often under genetic control (Li and Foley 1997; Foley and Fennimore 1998; Gu and others 2004), and a loss of genetic diversity in seed germination timing can negatively impact the ability of produced seeds to establish in a variety of restoration conditions (Kalisz 1986). The differences in germination timing and conditions between individuals serve as a form of bet-hedging to ensure some seedlings will survive regardless of seasonal fluctuations and microsite variation. Producing plant material with a range of germination timing (and therefore genetic diversity) will increase the chances that at least some of the plants are able to survive and grow in a restoration. For example, plants that germinate early will be more susceptible to late-spring killing frosts but, if they survive, will also be larger and more competitive than late-germinating plants.

### Application

1. Sow different populations separately.
2. Use germination conditions that maximize germination of as many seeds as possible, and do not favor seeds that germinate faster or under certain conditions more than others.
3. Keep germination flats for as long as possible and consider multiple cycles of stratification.

---

**RULE 7. Lessen the impacts of plant maintenance.** Establish growing conditions for each seedlot that encourage representation of all collected plants in produced material. If high mortality occurs, replant production beds or seed orchards using material from the original source population.

---

### Background

When plants are brought into production, some individuals will be more amenable to cultivation than others. Growing conditions under cultivation are often very different from those found in the wild. Cultivation provides plants with a relatively stress-free environment and allows the production of healthy plants. Studies of crop species suggest that, over time, plants brought in to cultivation diverge from their wild ancestors in several traits, including seed retention, increased fruit or seed size, changes in stature, disease resistance, change in reproductive strategy, and even changes in secondary metabolites (Harlan 1971). These changes have been defined as the *domestication syndrome*.

The period of plant maintenance within a nursery or production bed has high potential to lead to adaptation to cultivation that will diminish genetic variability (Havens and others 2004). As discussed in Rule 6, germination conditions can favor some individuals over others, but the influence of cultivation does not stop there. Plants slower to establish, grow, flower, and produce seeds are more likely to be lost or excluded from the production process. An example comes from the common sunflower (*Helianthus annuus* L. [Asteraceae]) growing as weeds in agricultural crop settings. Over multiple generations, plants that responded well to the growing conditions had lower drought tolerance, increased susceptibility to fungal infections, and were more palatable to insects (Mayrose and others 2011).

Another important genetic concern when native plants are brought into cultivation is that the local environmental and biological conditions at production sites will inevitably differ from the wild populations. Just as plants can adapt to cultivation, they may also adapt to local growing conditions at the production site. The larger the variations between collection site and production site, the greater the potential impact on the genetic diversity of plants produced. High mortality associated with differences in local growing conditions can result in loss of important genetic diversity (Knapp and Rice 1994). For example, exposure to new or high densities of pests and diseases during production can result in high loss of individuals (Altizer and others 2003). Plant loss due to diseases and pests in cultivated conditions are among the leading causes of accidental loss of diversity in many crops systems (Barrett 1981). To minimize the chance that plant mortality in seed production beds or seed orchards will negatively affect the genetic diversity of produced material, replant as needed with appropriate source material if attrition is high. Note that attrition may not be obvious in all situations. For example, attrition may be masked in perennial plants that self-sow or reproduce clonally.

### Application

1. Grow plants in conditions that maximize survival and seed production of all plants in a seedlot.
   a. Modify conditions of light, water, nutrient supplementation, pruning, soil type, and pest and disease control as needed to maximize survival of all individuals in each seedlot.
2. Do not continue to use production beds or seed orchards if plant loss is high.
   a. Ensure that clonal growth or self-sowing does not obscure plant attrition over time.

---

**RULE 8. Minimize unintended hybridization.** Use production methods to avoid crossing between seedlots.

---

### Background

An important prerequisite of seed production is successful pollination. For many species, this requires pollinators that move pollen from one plant to another. Pollination between individuals within a population creates genetically diverse seeds that are valuable to restoration practitioners. Many production facilities, however, work with multiple species or even multiple populations of each species. Consequently, populations or species that were once isolated by large distances may now be in close contact in a production field. This close proximity can increase the likelihood of unwanted hybridization between populations and even species.

While hybridization between seedlots may increase genetic diversity in the produced seeds, this may not always be advantageous for a restoration. Plants growing in production beds and seed orchards near each other may cross-pollinate with other species or populations, unintentionally diluting the genes that made that population unique. This may change the genetic variability of produced seeds and introduce the possibility that genes from one population may override those from another population, a process called *genetic swamping* (see Example 3) (Hufford and Mazer 2003). In extreme cases, hybridization between seedlots may lead to a failure to produce viable seeds (Baack 2005; Frankham and others 2011).

Options to prevent cross-pollination among seedlots in production include temporal or spatial separation of seedlots. The distance required for spatial separation varies by species. Spatial separation is a minimal concern for species that are primarily self-pollinating, including grasses such as blue wildrye (*Elymus glaucus* Buckley [Poaceae]). For species that require cross-pollination (either by insects or by wind) to produce seeds, production beds containing different seedlots of the same species may need to be physically separated from each other by as much as 100 m (300 ft) for animal-pollinated species (Van Rossum and others 2011) and greater than 200 m (600 ft) for wind-pollinated species (Robledo-Arnuncio and Gil 2005). Spatial isolation can be augmented with barriers such as hedgerows. By maintaining isolated seedlots, it is possible to maintain the unique genetic diversity in each population.

### EXAMPLE 3. GENETIC SWAMPING DUE TO CROSSING BETWEEN SPECIES

An extreme example of genetic swamping comes from a rare species of lantana native to Florida (*Lantana depressa* Small [Verbenaceae]) (Figure 7) that is losing its genetic identity to one of its introduced horticultural relatives (*Lantana camara* L.) (Maschinski and others 2010). *Lantana camara* is a popular horticultural plant that has been planted near, but also escaped into, many of the sites where *L. depressa* grows. Unfortunately, these 2 species are able to cross-pollinate, and now pollen from *L. camara* is crossing with *L. depressa* plants and producing viable seeds, leading to the production of plants that are hybrids between the 2 species. Because *L. depressa* populations are small, these hybrid plants compete for resources and will continue to cross-pollinate with the native species. Over time it is expected that, without management to remove hybrids, all *L. depressa* plants in all populations will be hybrids that no longer contain unique genetic diversity.

### Application

1. Avoid cross-pollination among seedlots through either temporal or spatial separation.
   a. Grow seedlots of the same species at different times, or
   b. Maintain the maximum spatial separation feasible among seedlots and species that are known to cross-pollinate (including wildflowers that are pollinated by insects or grasses and trees that are pollinated by wind).

---

**RULE 9. Vary timing of seed harvests.** Use methods that maximize the number of plants represented and ensure that potential genetic diversity is not unintentionally left out.

---

### Background

Seeds do not mature uniformly within a population. This variation can be attributable to genetic differences or to small differences in sunlight, fertilizer, or irrigation patterns in field plots. Harvesting seeds within large production areas is often done by machinery and during a very narrow time frame. Seed harvest is often timed around peak ripening to ensure most seeds will be at the right stage. Unfortunately, there will always be a subset of seeds that have not yet ripened, or that have already dispersed; this usually represents the earliest and latest flowering individuals, or those that are less prone to shatter and ripen during a shorter period of time. Any differences in seed maturation or release timing may lead to a loss of genetic diversity if they are not incorporated into seed harvest timing and approach. Harvest characteristics such as seed shatter are genetically controlled, so seeds that may be easier to harvest may be genetically different from seeds that are harder to harvest (Cai and Morishima 2000). As discussed in Rule 3, harvesting at peak maturity, and harvesting multiple times whenever possible, will help maintain genetic variation within produced material.

### Application

1. Harvest seeds at peak maturity and, if possible, harvest multiple times.
2. Modify harvest technique with each population. Seed size and shape will determine which tools will collect the maximum number of diverse seeds.

**RULE 10. Limit the number of generations during which plants are exposed to controlled production conditions.** Genetic loss and change (adaptation to cultivation) can occur during just one generation, and this may lead to plant material poorly suited to restoration conditions.

### Background

Seeds that are adapted to cultivated conditions are of limited value to restoration practitioners if they are not able to survive the stresses of a restoration site (Schröder and Prasse 2013). As highlighted in Rules 6 to 9, cultivation often favors some individuals over others. With each generation grown in cultivation, seedlots are increasingly likely to lose genetic diversity and to become adapted to cultivation conditions. These genetic shifts can occur in just one generation (Stanford and others 1960) and can become quite pronounced after multiple generations (Hoskinson and Qualset 1967). Therefore, if the goal is to produce seeds that contain genetic diversity and adaptation as similar to the original collection as possible, nursery-grown plants, production beds, and seed orchards should preferably be established using wild-collected seeds (Knapp and Rice 1994). This approach is not always feasible or implemented in practice, particularly for seedlots that are small and (or) are in high demand for restoration, situations wherein first- or second-generation seeds are often used (Shaw and others 2005). The Association of Official Seed Certifying Agencies may certify seedlots of native plants grown for up to 5 generations in cultivation, but the number of generations must be included on the seed label (AOSCA 2003).

Additionally, we know from Rule 2 that genetic diversity is lost in small populations over time because of random effects (genetic drift). These random processes will also diminish genetic variability in production beds. Even if a production field begins with a genetically representative sample, each production generation will lose variability due to drift (Schoen and Brown 2001). This loss may be mitigated by initially growing out a large number of unrelated individuals, but each additional generation in production leads to more interbreeding, making it increasingly unlikely that the full genetic diversity present in the original collection still remains (Figure 8).

*Figure 7.* Golden lantana (*Lantana depressa*), a species subject to extreme genetic swamping. Photo by Keith A Bradley

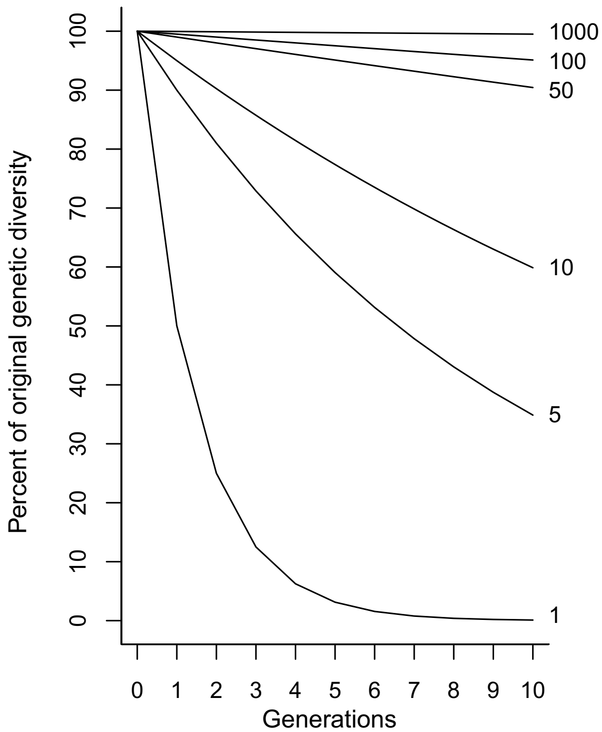


*Figure 8.* Initial genetic diversity lost over generations for a range of population sizes (Frankham and others 2010). A population of 1000 individuals will lose negligible genetic diversity after 10 generations, while a population of 50 will lose about 10% of its genetic diversity, and a population of 10 will lose 40% of its genetic diversity over the same amount of time.

### Application

1. To most effectively maintain the genetic diversity of the original collection and minimize adaptation to cultivation, use only wild-collected material when developing production beds, seed orchards, or producing plant material in a nursery.
2. Limit the number of generations that a seedlot is produced in cultivation.
3. For any grow out, ensure that large sample sizes are used and that every effort is made to minimize unintended selection.

## CONCLUSIONS AND RECOMMENDATIONS

The potential for genetic diversity to be lost during the production of native plant material exists at every step of the cultivation process, from seed collection through restoration planting. Every population contains a spectrum of desirable qualities, and each of the rules described here is intended to help maintain as much unique genetic information as possible throughout the production process. While each of the steps outlined above may not be appropriate or feasible in every situation, integrating these rules and applications into the propagation cycle will help maintain the maximum amount of genetic diversity. Some of these characteristics may not be conducive to mechanized or rigorous production practices. Nonetheless, with careful consideration, the economic realities of plant production can be balanced with the genetic necessities of successful restoration projects.

Each restoration site will pose unique challenges for installed plant material, and a changing climate will alter these needs in unforeseen ways (Havens and others 2015). The best way to prepare for these challenges is to ensure the availability of genetically diverse native plant materials with detailed notes on source conditions and propagation steps. With this information, restoration practitioners will have the capability to choose the most appropriate material for their site.

Only a handful of studies have assessed how production processes influence the genetic diversity of restored populations. One study of a tallgrass prairie restoration in Indiana found that the genetic diversity of restoration sites can be similar to wild populations when the production rules described here are followed (Dolan and others 2008). In the referenced study, all but the rarest genetic diversity was successfully maintained throughout production. The opposite result was found in a study of American beachgrass (*Ammophila breviligulata* Fernald [Poaceae]) restorations in the Great Lakes region. For this species, restorations using seedlings from clonally propagated material rather than grown from genetically diverse seed collections led to restorations with lower genetic diversity than what was found in the natural populations (Fant and others 2008). This result is particularly concerning given recent research showing that populations of American beachgrass with higher genetic diversity have better colonization success (Crawford and Whitney 2011). Finally, a study of beech trees in Europe (*Fagus sylvatica* L. [Fagaceae]) showed that seed collection (Rules 1–3 here), rather than nursery production (Rules 4–10), presented the greatest limiting step in maximizing and maintaining genetic diversity (Konnert and Ruetz 2003). Summarily considered, recent studies highlight how propagation techniques can have an important impact on the amount of genetic diversity that ends up at restoration sites, although additional research is still needed to help identify which steps pose the highest risk to the loss of genetic diversity. By working together, native plant material producers and researchers can identify factors of potential genetic loss in production situations to continually refine these rules and best practices to collect and maintain genetic diversity.

### Glossary

**Adaptation:** the genetic changes of a group of individuals over multiple generations in response to surrounding environmental or biological pressures.

**Genetic diversity:** the amount and distribution of genetic information within a population. This can be viewed as the number of variants of a gene present, the way in which these variants are organized within an individual, or the way in which individuals differ from one another.

**Genetic drift:** the change in genetic diversity within a population resulting from chance. These random losses of individuals can be attributable to lack of reproduction or death.

**Genetic swamping:** the loss of local genetic diversity in a population because of the introduction of large numbers of nonlocal individuals.

**Population:** a group of individuals growing close enough together that any 2 plants could likely interbreed. Some stands of plants may be easy to delineate as a definitive population. If a species has extensive pollen or seed dispersal, however, a population may be larger than a visual stand of plants. This is particularly true of species with wind- or animal-pollination as well as wind-dispersed seeds.

**Seed transfer zone:** geographic regions where plant populations are likely adapted to similar environmental conditions. Collecting and planting plant material within the same zone will minimize the risk of poor performance due to lack of adaptation.

**Numerous additional resources on this subject are available, including:**

Dunwiddie P, Delvin E. 2006. Inadvertent selection in the propagation of native plants: a cautionary note. Native Plants Journal 7(2):121–124.

Smith SE, Halbrook K. 2004. A plant genetics primer: basic terminology. Native Plants Journal 5(2):105–111.

USDA Forest Service. *Why We Care about Genetics* fact sheets, particularly volume 10: *Can Genetic Diversity be Influenced by Nursery Practices?* URL: http://www.fs.fed.us/wildflowers/Native_Plant_Materials/genetics/ (accessed 14 Sep 2014).

## ACKNOWLEDGMENTS

Many thanks to the organizers of the National Native Seed Conference for providing the impetus for developing these rules; to Stuart Wagenius for assistance with figures; to Keith A Bradley, Sheree Pickens, and Nora Talkington for the use of their photos; and to Ben Alexander, Mel Asher, Jerry Benson, Kas Dumroese, Maggie Eshleman, Jessa Finch, Alicia Foxx, Ed Guerrant, Kayri Havens, Joan O'Shaughnessy, Kelly Schultz, David Sollenberger, Nora Talkington, Christopher Warneke, and 3 anonymous reviewers for thoughtful comments on previous versions of this manuscript.

## REFERENCES

Ågren J, Schemske DW. 2012. Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. New Phytologist 194(4):1112–1122.

Altizer S, Harvell D, Friedle E. 2003. Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology & Evolution 18(11):589–596.

[AOSCA] Association of Official Seed Certifying Agencies. 2003. The AOSCA Native Plant Connection. URL: http://www.aosca.org/SiteContent/Documents/aoscanativeplantbrochure.pdf (accessed 8 Jan 2015).

Baack EJ. 2005. Ecological factors influencing tetraploid establishment in snow buttercups (*Ranunculus adoneus*, Ranunculaceae): minority cytotype exclusion and barriers to triploid formation. American Journal of Botany 92(11):1827–1835.

Barrett JA. 1981. The evolutionary consequences of monoculture. In: Bishop JA, Cook LM, editors. Genetic consequences of man-made change. London: Academic Press. p 209–246.

Baskin CC, Baskin JM. 2001. Seeds: ecology, biogeography, and evolution of dormancy and germination. San Diego (CA): Academic Press. 666 p.

Bennington CC, Fetcher N, Vavrek MC, Shaver GR, Cummings KJ, McGraw JB. 2012. Home site advantage in two long-lived arctic plant species: results from two 30-year reciprocal transplant studies. Journal of Ecology 100(4):841–851.

Bonner F. 2003. Collection and care of acorns: a practical guide for seed collectors and nursery managers. v 1.1. 28 p. URL: http://www.nsl.fs.fed.us/collection%20and%20care%20of%20acorns.pdf (accessed 8 Jan 2015).

Bonner FT, Karrfalt RP, editors. 2008. The woody plant seed manual. Agriculture Handbook 727. URL: http://www.nsl.fs.fed.us/nsl_wpsm.html (accessed 8 Jan 2015). Washington (DC): USDA Forest Service.

Bower AD, St Clair JB, Erickson V. 2014. Generalized provisional seed zones for native plants. Ecological Applications 24:913–919.

Breed MF, Stead MG, Ottewell KM, Gardner MG, Lowe AJ. 2013. Which provenance and where? Seed sourcing strategies for revegetation in a changing environment. Conservation Genetics 14(1):1–10.

Broadhurst LM, Lowe A, Coates DJ, Cunningham SA, McDonald M, Vesk PA, Yates C. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. Evolutionary Applications 1(4):587–597.

Cabin RJ, Evans AS, Mitchell RJ. 1997. Genetic effects of germination timing and environment: an experimental investigation. Evolution 51(5):1427–1434.

Cai HW, Morishima H. 2000. Genomic regions affecting seed shattering and seed dormancy in rice. Theoretical and Applied Genetics 100(6):840–846.

Clewell A, Aronson J. 2013. The SER primer and climate change. Ecological Management & Restoration 14(3):182–186.

Conesa HM, García G, Faz Á, Arnaldos R. 2007. Dynamics of metal tolerant plant communities' development in mine tailings from the Cartagena-La Unión Mining District (SE Spain) and their interest for further revegetation purposes. Chemosphere 68(6):1180–1185.

Corre VL. 2005. Variation at two flowering time genes within and among populations of *Arabidopsis thaliana*: comparison with markers and traits. Molecular Ecology 14(13):4181–4192.

Crawford KM, Rudgers JA. 2012a. Genetic diversity within a dominant plant outweighs plant species diversity in structuring an arthropod community. Ecology 94(5):1025–1035.

Crawford KM, Rudgers JA. 2012b. Plant species diversity and genetic diversity within a dominant species interactively affect plant community biomass. Journal of Ecology 100(6):1512–1521.

Crawford KM, Whitney KD. 2011. Population genetic diversity influences colonization success. Molecular Ecology 19(6):1253–1263.

Crossa J, Vencovsky R. 2011. Basic sampling strategies: theory and practice. In: Collecting plant genetic diversity: technical guidelines. 28 p. URL: http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=671 (accessed 8 Jan 2015).

Dalling JW, Hubbell SP. 2002. Seed size, growth rate and gap microsite conditions as determinants of recruitment success for pioneer species. Journal of Ecology 90(3):557–568.

Doede DL. 2005. Genetic variation in broadleaf lupine (*Lupinus latifolius*) on the Mt Hood National Forest and implications for seed collection and deployment. Native Plants Journal 6(1):36–48.

Dolan RW, Marr DL, Schnabel A. 2008. Capturing genetic variation during ecological restorations: an example from Kankakee Sands in Indiana. Restoration Ecology 16(3):386–396.

Duminil J, Fineschi S, Hampe A, Jordano P, Salvini D, Vendramin GG, Petit RJ. 2007. Can population genetic structure be predicted from life-history traits? American Naturalist 169(5):662–672.

Ensslin A, Sandner TM, Matthies D. 2011. Consequences of *ex situ* cultivation of plants: genetic diversity, fitness and adaptation of the monocarpic *Cynoglossum officinale* L. in botanic gardens. Biological Conservation 144(1):272–278.

Fahnestock JT, Detling JK. 2000. Morphological and physiological responses of perennial grasses to long-term grazing in the Pryor Mountains, Montana. American Midland Naturalist 143(2):312–320.

Falk DA, Knapp EE, Guerrant EO. 2001. Introduction to restoration genetics. URL: http://www.ser.org/docs/default-document-library/ser_restoration_genetics.pdf?sfvrsn=0 (accessed 8 Jan 2015). Society for Ecological Restoration. 33 p.

Fant JB, Holmstrom RM, Sirkin E, Etterson JR, Masi S. 2008. Genetic structure of threatened native populations and propagules used for restoration in a clonal species, American beachgrass (*Ammophila breviligulata* Fern.). Restoration Ecology 16(4):594–603.

Fant JB, Havens K, Keller JM, Radosavljevic A, Yates ED. 2014. The influence of contemporary and historic landscape features on the genetic structure of the sand dune endemic, *Cirsium pitcheri* (Asteraceae). Heredity 112(5):519–530.

Ferrero-Serrano Á, Hild AL, Mealor BA. 2011. Can invasive species enhance competitive ability and restoration potential in native grass populations? Restoration Ecology 19(4):545–551.

Foley ME, Fennimore SA. 1998. Genetic basis for seed dormancy. Seed Science Research 8(2):173–182.

Frankham R, Ballou J, Briscoe D. 2010. Introduction to conservation genetics. 2nd ed. Cambridge: Cambridge University Press. 617 p.

Frankham R, Ballou JD, Eldridge MDB, Lacy RC, Ralls K, Dudash MR, Fenster CB. 2011. Predicting the probability of outbreeding depression. Conservation Biology 25(3):465–475.

Gibbs JP, Smart LB, Newhouse AE, Leopold DJ. 2012. A molecular and fitness evaluation of commercially available versus locally collected blue lupine *Lupinus perennis* L. seeds for use in ecosystem restoration efforts. Restoration Ecology 20(4):456–461.

Gu XY, Kianian SF, Foley ME. 2004. Multiple loci and epistases control genetic variation for seed dormancy in weedy rice (*Oryza sativa*). Genetics 166(3):1503–1516.

Guerrant EO, Havens K, Maunder M. 2004. Ex situ plant conservation: supporting species survival in the wild. Washington (DC): Island Press. 504 p.

Hale ML, Burg TM, Steeves TE. 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLoS ONE 7(9):e45170.

Hare JD. 1980. Variation in fruit size and susceptibility to seed predation among and within populations of the cocklebur, *Xanthium strumarium* L. Oecologia 46(2):217–222.

Harlan JR. 1971. Agricultural origins: centers and noncenters. Science 174:468–474.

Harris JA, Hobbs RJ, Higgs E, Aronson J. 2006. Ecological restoration and global climate change. Restoration Ecology 14(2):170–176.

Havens K, Guerrant EO, Maunder M, Vitt P. 2004. Guidelines for *ex situ* conservation collection management: minimizing risks. In: Guerrant EO, Havens K, Maunder M, editors. Ex situ plant conservation: supporting species survival in the wild. Washington (DC): Island Press. p 454–473.

Havens K, Vitt P, Still S, Kramer A, Fant JB, Schatz K. 2015. Seed sourcing for restoration in an era of climate change. Natural Areas Journal 35(1): forthcoming.

Herman B, Packard S, Pollack C, Houseal G, Sinn S, O'Leary C, Fant J, Lewis AD, Wagenius S, Gustafson D. 2014. Decisions . . . decisions . . . how to source plant material for native plant restoration projects. Ecological Restoration 32(3):236–238.

Hoban S, Schlarbaum S. 2014. Optimal sampling of seeds from plant populations for *ex-situ* conservation of genetic biodiversity, considering realistic population structure. Biological Conservation 177:90–99.

Hoskinson PE, Qualset CO. 1967. Geographic variation in Balboa rye. 62(2):8–9.

Hufford K, Mazer SJ. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology & Evolution 18(3):147–155.

Hughes AR, Stachowicz JJ. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proceedings of the National Academy of Sciences 101(24):8998–9002.

Johnson GR, Sorensen FC, St Clair JB, Croon RC. 2004. Pacific Northwest forest tree seed zones: a template for native plants? Native Plants Journal 5(2):131–140.

Johnson GR, Stritch L, Olwell P, Lambert S, Horning ME, Cronn R. 2010. What are the best seed sources for ecosystem restoration on BLM and USFS lands? Native Plants Journal 11(2):117–131.

Johnson MTJ, Agrawal AA. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). Ecology 86(4):874–885.

Johnson RC, Cashman MJ, Vance-Borland K. 2012. Genecology and seed zones for Indian ricegrass collected in the southwestern United States. Rangeland Ecology & Management 65(5):523–532.

Jump AS, Marchant R, Peñuelas J. 2009. Environmental change and the option value of genetic diversity. Trends in Plant Science 14(1):51–58.

Kalisz S. 1986. Variable selection on the timing of germination in *Collinsia verna* (Scrophulariaceae). Evolution 40(3):479–491.

Karron JD, Holmquist KG, Flanagan RJ, Mitchell RJ. 2009. Pollinator visitation patterns strongly influence among-flower variation in selfing rate. Annals of Botany 103(9):1379–1383.

Kitzmiller JH. 1990. Managing genetic diversity in a tree improvement program. Forest Ecology and Management 35(1–2):131–149.

Knapp EE, Rice KJ. 1994. Starting from seed: genetic issues in using native grasses for restoration. Restoration & Management Notes 12(1):40–45.

Konnert M, Ruetz W. 2003. Influence of nursery practices on the genetic structure of beech (*Fagus sylvatica* L.) seedling populations. Forest Ecology and Management 184(1–3):193–200.

Kramer A, Larkin D, Fant JB. 2015. Assessing potential seed transfer zones for five forb species in the Great Basin. Natural Areas Journal 35(1):forthcoming.

Kulpa SM, Leger EA. 2013. Strong natural selection during plant restoration favors an unexpected suite of plant traits. Evolutionary Applications 6(3):510–523.

Lawrence MJ, Marshall DF, Davies P. 1995. Genetics of genetic conservation. I. Sample size when collecting germplasm. Euphytica 84(2):89–99.

Leger EA. 2008. The adaptive value of remnant native plants in invaded communities: an example from the Great Basin. Ecological Applications 18(5):1226–1235.

Leimu R, Fischer M. 2008. A meta-analysis of local adaptation in plants. PLoS ONE 3(12):e4010.

Leimu R, Mutikainen P, Koricheva J, Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology 94(5):942–952.

Lesica P, Allendorf FW. 1999. Ecological genetics and restoration of plant communities: mix or match? Restoration Ecology 7:42–50.

Li B, Foley ME. 1997. Genetic and molecular control of seed dormancy. Trends in Plant Science 2(10):384–389.

Linhart YB, Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics 27:237–277.

Love SL, Tripepi RR, Salaiz T. 2014. Influence of harvest timing and storage interval on rabbitbrush seed germination, emergence, and viability. Native Plants Journal 15(2):98–108.

Luna T, Wilkinson KM. 2009. Collecting, processing, and storing seeds. In: Dumroese RK, Luna T, Landis TD, editors. Nursery manual for native plants: guide for tribal nurseries. Volume 1: Nursery management. Agriculture Handbook 730. Washington (DC): USDA Forest Service. p 113–131.

MacKay J. 1993. Seed source selection and genetic improvement of red oak (*Quercus rubra* L.) in Québec. Annales des sciences forestières 50(Supplement):420s–424s.

Mahalovich MF, McArthur ED. 2004. Sagebrush (*Artemisa* spp.) seed and plant transfer guidelines. Native Plants Journal 5(2):149–151.

Maschinski J, Haskins KE. 2012. Plant reintroduction in a changing climate: promises and perils. Washington (DC): Island Press. 432 p.

Maschinski J, Sirkin E, Fant J. 2010. Using genetic and morphological analysis to distinguish endangered taxa from their hybrids with the cultivated exotic pest plant *Lantana strigocamara* (syn: *Lantana camara*). Conservation Genetics 11:1607–1621.

Mayrose M, Kane NC, Mayrose I, Dlugosch KM, Rieseberg LH. 2011. Increased growth in sunflower correlates with reduced defences and altered gene expression in response to biotic and abiotic stress. Molecular Ecology 20(22):4683–4694.

McKay JK, Christian CE, Harrison S, Rice KJ. 2005. "How local Is local?" A review of practical and conceptual issues in the genetics of restoration. Restoration Ecology 13(3):432–440.

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

Montalvo A, Rice SLW, Cory SLBC, Handel SN, Nabhan GP, Primack R, Robichaux RH. 1997. Restoration biology: a population biology perspective. Restoration Ecology 5(4):277–290.

Obeso JR, Martínez I, García D. 2011. Seed size is heterogeneously distributed among destination habitats in animal dispersed plants. Basic and Applied Ecology 12(2):134–140.

Rees M. 1996. Evolutionary ecology of seed dormancy and seed size. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences 351(1345):1299–1308.

Reusch TBH, Ehlers A, Hammerli A, Worm B. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proceedings of the National Academy of Sciences 102(2):2826–2831.

Reynolds LK, McGlathery KJ, Waycott M. 2012. Genetic diversity enhances restoration success by augmenting ecosystem services. PLoS ONE 7(6):e38397.

Rhodes MK, Fant JB, Skogen KA. 2014. Local topography shapes fine-scale spatial genetic structure in the Arkansas Valley Evening Primrose, *Oenothera harringtonii* (Onagraceae). Journal of Heredity 105(6):806–815.

Robledo-Arnuncio JJ, Gil L. 2005. Patterns of pollen dispersal in a small population of *Pinus sylvestris* L. revealed by total-exclusion paternity analysis. Heredity 94(1):13–22.

Rogers DL. 2004. Genetic erosion: no longer just an agricultural issue. Native Plants Journal 5(2):113–122.

Rowe CLJ, Leger EA. 2011. Competitive seedlings and inherited traits: a test of rapid evolution of *Elymus multisetus* (big squirreltail) in response to cheatgrass invasion. Evolutionary Applications 4(3):485–498.

Saari C, Glisson W. 2012. Survey of Chicago region restoration seed source policies. Ecological Restoration 30(3):162–165.

Schoen DJ, Brown AHD. 2001. The conservation of wild plant species in seed banks. BioScience 51(11):960–966.

Schröder R, Prasse R. 2013. From nursery into nature: a study on performance of cultivated varieties of native plants used in re-vegetation, their wild relatives and evolving wild x cultivar hybrids. Ecological Engineering 60:428–437.

Shaw NL, Lambert SM, DeBolt AM, Pellant M. 2005. Increasing native forb seed supplies for the Great Basin. In: Dumroese RK, Riley LE, Landis TD, technical coordinators. National proceedings: Forest and Conservation Nursery Associations; 2004. Fort Collins (CO): USDA Forest Service, Rocky Mountain Research Station. Proceedings RMRS-P-35. p 94-105. URL: http://www.fs.fed.us/rm/pubs/rmrs_p035/rmrs_p035_094_102.pdf (accessed 8 Jan 2015).

Silen RR, Osterhaus C. 1979. Reduction of genetic base by sizing of bulk Douglas-fir seedlots. Tree Planters' Notes 30:24–30.

Smith SL, Sher AA, Grant TA. 2007. Genetic diversity in restoration materials and the impacts of seed collection in Colorado's restoration plant production industry. Restoration Ecology 15(3):369–374.

Stanford EH, Laude HM, Enloe JA. 1960. Effect of harvest dates and location on the genetic composition of the syn1 generation of Pilgrim Ladino Clover. Agronomy Journal 52(3):149–152.

Thompson PA. 1981. Variations in seed size within populations of *Silene dioica* (L.) Clairv. in relation to habitat. Annals of Botany 47(5):623–634.

Tooker JF, Frank SD. 2012. Genotypically diverse cultivar mixtures for insect pest management and increased crop yields. Journal of Applied Ecology 49(5):974–985.

Upadhyaya H, Kumar S, Gowda CLL, Singh S. 2006. Two major genes for seed size in chickpea (*Cicer arietinum* L.). Euphytica 147(3):311–315.

[USDA NRCS] USDA Natural Resources Conservation Service. 2015. The

PLANTS database. URL: http://plants.usda.gov (accessed 12 Apr 2014). Greensboro (NC): National Plant Data Team.
[USDI BLM] USDI Bureau of Land Management. 2012. Technical protocol for the collection, study, and conservation of seeds from native plant species for seeds of success. 39 p. URL: http://www.blm.gov/sos (accessed 8 Jan 2015).
Van Rossum F, Stiers I, Van Geert A, Triest L, Hardy O. 2011. Fluorescent dye particles as pollen analogues for measuring pollen dispersal in an insect-pollinated forest herb. Oecologia 165(3):663–674.
Vander Mijnsbrugge K, Bischoff A, Smith B. 2010. A question of origin: where and how to collect seed for ecological restoration. Basic and Applied Ecology 11(4):300–311.
Vekemans X, Hardy OJ. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. Molecular Ecology 13(4):921–935.
Vogler DW, Kalisz S. 2001. Sex among the flowers: the distribution of plant mating systems. Evolution 55(1):202–204.
Walters C, Wheeler LM, Grotenhuis JM. 2005. Longevity of seeds stored in a genebank: species characteristics. Seed Science Research 15(01):1–20.
Walters C, Berjak P, Pammenter N, Kennedy K, Raven P. 2013. Preservation of recalcitrant seeds. Science 339(6122):915–916.
Ward K, Gisler M, Fiegener R, Young A. 2008. The Willamette Valley Seed Increase Program: developing genetically diverse germplasm using an ecoregion approach. Native Plants Journal 9(3):334–350.
Withrow-Robinson BA, Johnson R. 2006. Selecting native plant materials for restoration projects: ensuring local adaptation and maintaining genetic diversity. Corvallis (OR): Oregon State University Extension Service. URL: http://ir.library.oregonstate.edu/xmlui/handle/1957/20385 (accessed 8 Jan 2015).
Youtie B, Shaw NL, Fisk M, Jensen S. 2012. A strategy for maximizing native plant material diversity for ecological restoration, germplasm conservation and genecology research. In: 8th European Conference on Ecological Restoration. České Budějovice, Czech Republic. 4 p. URL: http://www.fs.fed.us/rm/pubs_other/rmrs_2012_youtie_b001.pdf (accessed 8 Jan 2015).

## AUTHOR INFORMATION

**Adrienne C Basey**
Master's Candidate
Program in Plant Biology and Conservation
Biological Sciences, Weinberg College of Arts and Sciences
Northwestern University
Evanston IL 60208
adrienne.basey@u.northwestern.edu

**Jeremie B Fant**
Conservation Scientist
jfant@chicagobotanic.org

**Andrea T Kramer**
Conservation Scientist
akramer@chicagobotanic.org

Chicago Botanic Garden
Department of Plant Science and Conservation
1000 Lake Cook Road
Glencoe IL 60022