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Author(s): Edward O. Guerrant Jr., Kayri Havens and Pati Vitt,
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SAMPLING FOR EFFECTIVE EX SITU PLANT CONSERVATION

Edward O. Guerrant Jr.,[1],* Kayri Havens,† and Pati Vitt‡

*Rae Selling Berry Seed Bank & Plant Conservation Program, Department of Environmental Science and Management, Portland State University, P.O. Box 751-ESM, Portland, Oregon 97207, USA; †Division of Plant Biology and Conservation, Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, Illinois 60022, USA; and ‡Dixon National Tallgrass Prairie Seed Bank, Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, Illinois 60022, USA

Sampling a whole flora or any significant subset for ex situ conservation purposes is a complex, long-term proposition. Thus, it is important to consider what constitutes an adequate sample not only for all taxa as a whole but also for each taxon individually as well as how to strategically schedule collection over time. There are five basic sampling questions: from which species to collect, from how many and which populations, from how many and which individuals, how many and what kind of propagules to collect, and, finally, at what point is the desired sample size too great for a population to bear in one year? There is no single correct sampling strategy or protocol. Each situation must be evaluated in the context of the particular purposes, goals, and uses for which the collection is being made as well as the nature of the sampling universe, the manner in which the samples will be stored and maintained, the period of time they will be stored, and whether the wild-collected samples are to be used directly or whether their numbers will be increased by agricultural growouts. Purposes include providing a long-term "insurance policy" against extinction in the wild and, in the near to medium term, supplying native plant material for small- to large-scale restoration purposes. The term "sampling universe" refers to the target taxa of interest: it could be the entire seed plant flora of Taiwan or particular subsets, such as endemics, higher-elevation plants, ecologically important taxa, rare plants, and so on. Plants with orthodox seeds are much easier to work with and are more economically stored as dried, frozen seeds in a seed bank than either plants with recalcitrant seeds or taxa maintained as growing plants.

Keywords: climate change, ecological restoration, ex situ conservation, genetic sampling, seed banking.

Introduction

For millennia, people have stored seeds for future use. The practice presumably began with saving seeds of food plants, both to eat and to plant the next year. Saving seeds of agriculturally important plants is still the most widespread purpose served by seed banks. The science and practice of seed banking have made great progress in recent decades. Seed banks have also been increasingly applied to more diverse arrays of naturally occurring species, notably for conserving rare and common plants alike (Falk and Holsinger 1991; Falk et al. 1996; Australian Network for Plant Conservation Translocation Working Group 1997; Smith et al. 2003; Guerrant et al. 2004b; Maschinski and Haskins 2012).

Seed banking is now more important than ever. Species and populations are disappearing at a greatly accelerated rate as a result of habitat destruction, fragmentation, and isolation of suitable habitat as well as competition from invasive plant species. The effects of global climate change on biodiversity are likely to vastly increase this trend. Seed banking is a technically feasible and remarkably economical means to ensure

that the plant biodiversity we inherited is passed on to our descendants (Li and Pritchard 2009). We must act now, and do so effectively.

Seed banking takes advantage of the remarkable property many plants have that permits their seeds to be dried, frozen, and stored for long periods of time, after which they can germinate and grow to be healthy, reproductive adults. Seeds with this property are referred to as having "orthodox" seed storage behavior. Not all seeds can survive the desiccation and subsequent storage required by seed banking; ~12% of seed-bearing species have "recalcitrant" seeds (Hong et al. 1998). However, the science and practice of seed storage is expanding to include recalcitrant seeds as well as other seeds with so-called intermediate seed-storage behavior, which are also difficult to store (Smith et al. 2003). Significant progress is being made in the long-term banking of seeds with recalcitrant and intermediate storage behavior (Berjak and Pammenter 2008, 2014; Pammenter and Berjak 2014).

Given the magnitude and scope of the challenges we face in conserving plant diversity, seeds are the propagule of choice to collect and store, although there are instances where vegetative or other material must be used (Smith et al. 2003; Guerrant et al. 2004b). Seed banks offer the most cost-effective means of storing large numbers of species and individuals over long periods of time (Guerrant et al. 2004b; Li and Pritchard 2009). In creating a new seed bank or reassessing and ex-

[1] Author for correspondence; e-mail: guerran@pdx.edu.

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panding the mission of an existing seed bank, it is necessary to consider carefully the particular purpose or purposes stored samples are intended to serve.

With respect to collecting genetically representative samples, the five basic sampling questions outlined by the Center for Plant Conservation (1991) are relevant across a wide range of purposes and scales: (1) Which taxa should be sampled? (2) For each taxon, how many and which populations should be sampled? (3) Within each population, how many and which individuals should be sampled? (4) For each individual, how many and what type of propagules should be collected? (5) At what point does the target sample size exceed a population's ability to withstand collection (Menges et al. 2004) and endanger it? To these can be added the following: Will wild-collected material be directly used only to restore diversity to the wild, or will there be off-site cultivation of wild-collected material to increase seed numbers available for restoration? Ultimately, our choices of which species and populations to collect and how well we can store them for long periods of time may well mean that some plants survive into the next century that otherwise would not.

Answers for even the most basic questions, such as whether to collect from one or more populations of a taxon, depend on many factors, including the purposes for which the collections are to be made as well as the sampling universe from which they will be made. Different collection approaches have been used by different organizations. The Millennium Seed Bank (MSB; Royal Botanic Gardens, Kew, United Kingdom) and the Berry Botanic Garden Berry Seed Bank for Rare and Endangered Plants of the Pacific Northwest (now the Rae Selling Berry Seed Bank [RSBSB] at Portland State University, Portland, OR) provide examples on opposite ends of the spectrum in both their purpose and their scope. Encompassing extremes, the Chicago Botanic Garden's Dixon National Tallgrass Prairie Seed Bank (DNTPSB) has several conservation and restoration objectives with distinct sampling strategies for different categories of plants.

The MSB focuses on collecting a substantial portion of the global flora. The MSB achieved its initial goal of collecting samples from 10% of the world's seed plant taxa by 2010, and it is now working to collect samples from 25% of the world's seed plant taxa by the year 2020. The MSB collects a single large bulk collection, composed of seeds (~20,000) from many individuals from a single population. This maximizes taxonomic breadth and among-species diversity. Seed collection and storage at the MSB has grown and evolved. Originating with the horticulturally oriented index seminum at the Royal Botanic Gardens, Kew, in the United Kingdom, it next focused on wild collection in the dry tropics. It has become the core of a global partnership with the primary purpose of providing "extinction insurance" for the world's seed plants, particularly in support of the 2002 Global Strategy for Plant Conservation (GSPC; http://www.bgci.org/worldwide/gspc/). The GSPC provides 16 action-oriented targets to ensure long-term conservation of the global flora. The MSB focused on target 8, which aims to ensure inclusion of "60% of threatened plant species in accessible ex-situ collections, preferably in the country of origin and 10% of them included in recovery and restoration programs."

Early on, the MSB was criticized for targeting only a single (albeit large) accession of each taxon on the grounds that it did not capture the scope of genetic variation within each taxon. This is true as far as it goes, but the global flora presents a sampling universe in the hundreds of thousands, in which terminal taxa are the sampling unit of interest. At that scale, a single point-source sample will provide the overwhelming majority of genetic information in that terminal taxon. While this strategy comes at a cost of genetic diversity within a taxon, it still captures the vast majority of genetic information for each sampled taxon and maximizes genetic diversity among taxa for a given sampling effort.

At the other extreme and with a focus on rare and endangered species, the purpose of the RSBSB is to provide extinction insurance of sampled populations as well as material for possible reintroduction. The RSBSB focuses on a narrow geographic area, collecting seeds from separate maternal lines (collections made from each of many genetic individuals), from multiple populations across the range of each species, and ideally from the same population at different times within a single year as well as across different years (Center for Plant Conservation 1991; Guerrant 1996; Guerrant et al. 2004b). This strategy maximizes within-species genetic diversity. Neither approach is inherently superior to the other, as both are appropriate for their purposes and sampling universes.

Created within the last decade, the Chicago Botanic Garden's DNTPSB incorporates the best of both, utilizing distinct sampling approaches to meet each of several different purposes. The DNTPSB focuses its collecting efforts on species found within the tallgrass prairie region of the Midwestern United States (fig. 1). Its goals are threefold: (1) to collect and bank at least one representative sample of each species comprising the flora of the region from all habitats found therein, including prairies, savannas, woodlands, and wetlands; (2) to collect and bank multiple samples of the rare and threatened flora of the region; and (3) to collect and bank multiple genetically diverse samples of "matrix species," that is, those species that are most important to restoration efforts throughout the region.

In response to the increasingly clear threat of global climate change to biodiversity, the RSBSB is in the process of expanding its coverage to include common species as well as rare species. The core focal area of what will become the Oregon Ecoregions Seed Bank (OESB) is circumscribed by the biologically irrelevant political boundary of the state of Oregon (fig. 1). The actual area of interest is larger, comprising the full extent of any ecoregion level 3 that is found in Oregon. Note that the DNTPSB and the OESB, while both emphasizing an ecoregional approach, define their core areas very differently. The point is that there is no one correct way to circumscribe the focal area of a seed bank, and a variety of approaches are possible. Ideally, the country and world will eventually be comprehensively served a network of overlapping regional seed banks that collect multiple large samples of taxa across their ranges.

Our goal in this article is to explore what is involved in effective sampling for ex situ plant conservation. In addition to defining the purposes for which collections will be made, we offer decision-making recommendations about what species to select, from how many and which populations, and how much to collect. We approach the topic in general terms,
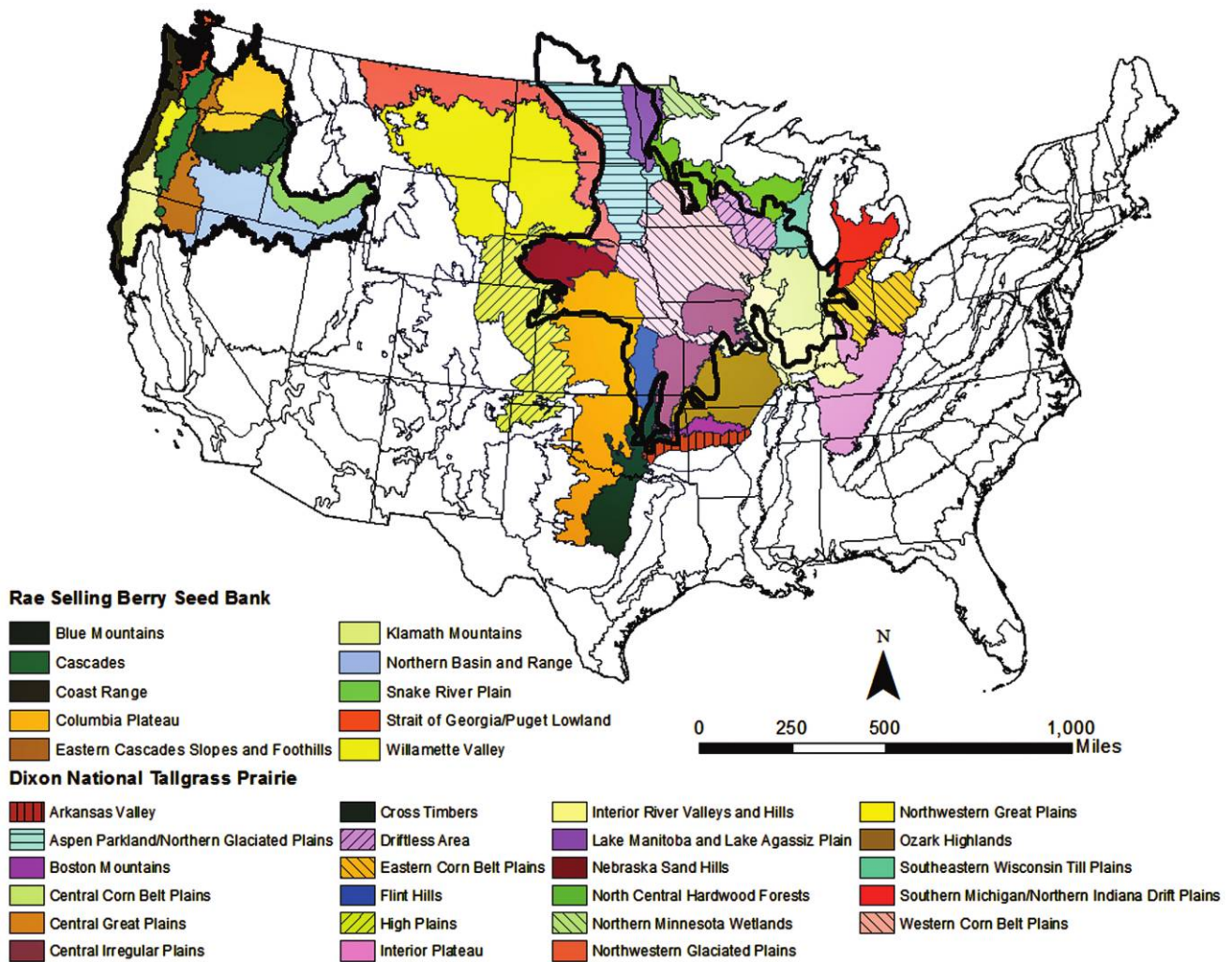
Fig. 1 Map illustrating the collecting regions of the Dixon National Tallgrass Prairie Seed Bank (DNTPSB) and the Oregon Ecoregions Seed Bank (OESB). The DNTPSB coverage is defined by the extent of the tallgrass prairies of the upper Midwest region of the United States, extending into Canada (outlined in black), as is illustrated against a backdrop of the relevant US Environmental Protection Agency level 3 ecoregions. The core OESB area of coverage is based in an arbitrarily defined political boundary, the state of Oregon, and extends to include the entire level 3 ecoregion of all that are found in Oregon. State and ecoregion level 3 boundaries for the rest of the continental United States are indicated as a geographic reference.

and we finish with a synthetic summary section that explores how the DNTPSB addressed issues common to conservation seed banks.

Seed Bank Purposes

In practice, seed banks commonly have multiple purposes that continue to evolve over time. Sampling an entire flora (or any significant subset) for ex situ conservation purposes is a long-term proposition spanning decades to centuries. Thus, it is essential to define the purpose or set of purposes for which the samples are collected. Of the wide range of possible purposes for undertaking the collection of seeds or other propagules, there are two not entirely mutually exclusive basic categories. At one extreme, the samples constitute a long-term hedge against loss of biodiversity, from genes through populations to species or even larger monophyletic groups. At the other extreme, propagules are collected as part of the supply chain for shorter-term ecological restoration or research efforts. The former purpose essentially provides an "insurance policy" against extinction, while the latter provides "working capital" for restoration efforts. In neither case are the samples a conservation end in themselves; rather, they are a means to an end: to enhance the prospects of survival of native taxa in the face of uncertain future environmental conditions. In practice, seed banks commonly have multiple purposes, which continue to evolve over time.

Long-Term Conservation

The stated purpose of a collection strongly influences the goals, strategies, and objectives necessary to support that pur-

pose. Long-term storage of naturally occurring taxa may be undertaken for a variety of reasons and may include wild relatives of crop species for use in breeding as well as species that are important for other human uses, such as medicine and fiber for clothing. It may also be undertaken to ensure the survival of the rarest species or to provide foundational material for native plant material development and large-scale ecological restoration efforts.

Restoration Material

Storing seed for ecological restoration covers a variety of situations, which may require different collection strategies. Material for ecological restoration includes a diverse array of purposes spanning a wide spectrum of geographic scales and timescales, not to mention focal taxa and the degree to which particular samples can be matched in advance of particular restoration projects. At one end, the purpose might be to respond to specific planned disruptions, the size and location of which are known in advance. Examples include highly localized short-term disruptions, such as road realignment projects or disturbances like prescribed burning, and larger long-term disruptions, such as mining. There are other large-scale disturbances that can be anticipated generally, such as wildfires and floods, but their specific locations, size, and timing cannot. Material intended for possible future assisted migration (also known as assisted colonization and managed relocation) may also be considered as part of a restoration mission.

Some of the anthropogenic disturbances that will only increase the need for future restoration include large-scale habitat conversion for energy development, including alternative energy sources like solar and wind farms. Increased pressure from hydroelectric projects may affect some of the unique flora in ravines and gorges, areas that historically have served as refugia. Proactive seed collection may be particularly important in these threatened habitats.

In our global society, another anthropogenic factor to consider is the threat from introduced pests and pathogens. We have seen drastic changes to natural landscapes when a new pest or pathogen arrives, such as the loss of the American chestnut (*Castanea dentata*) in the United States due to chestnut blight (*Cryphonectria parasitica*) and the effects of dieback caused by *Phytopthora cinnamomi* on many native species in Australia. If affected populations and species can be quickly targeted for seed collection before pests and diseases become widespread, there is a much higher likelihood of capturing germplasm for future breeding programs for resistance to the pest or future reintroductions after the pest has been controlled. Several regional projects in the United States are currently under way to bank seeds of ash trees (*Fraxinus* spp.) that are being decimated by the emerald ash borer (*Agrilus planipennis*). Similarly, other efforts are targeting eastern and Carolina hemlock (*Tsuga* spp.) seed collections in response to the hemlock woolly adelgid (*Adelges tsugae*).

Along with the material to be used directly in restoration efforts, it is necessary to have sufficient material to develop baseline information about the taxon (e.g., seed storage behavior) and for various propagation protocols (e.g., germination and establishment in the wild) and to monitor sample vigor and viability over time. In addition, a legitimate purpose

of seed banks is supplying material for other scientific studies, such as phylogenetic, ecological, ecogenetic, and common-garden experiments, which directly or indirectly benefit conservation.

Which Species to Bank?

In addition to purpose, taxonomic sampling universe and geographic scope set the boundaries for selecting species. Nevertheless, a great many criteria affect species choice, which can be grouped into four broad categories: geographic, phylogenetic, environmental and ecological, and utilitarian or cultural uses. These criteria are not mutually exclusive, and many seed banks may use some or all in developing an institutional strategy. Generally, the list of desired taxa and populations from which to collect are so great that it is necessary to spread collections over time, from several years to decades.

Geographic Scope

Most seed banks focused on native flora have a geographic area of focus, either as politically bounded regions (continents, countries, states) or ecologically cohesive regions (e.g., the tallgrass prairie flora or the flora of the Canary Islands). Geographic boundaries used to guide seed collection activities are often prescribed by easily established geopolitical boundaries at the national, provincial, or state level, as is the case for the RSBSB for the state of Oregon. Collection is not strictly limited to those arbitrary boundaries but extends outward to include surrounding areas that share ecoregions found in the state.

Phylogenetic History

The confluence of cladistic reasoning and an ability to generate DNA-level molecular genetic data have provided a virtual quantum leap in our understanding of the patterns of evolutionary relationships among organisms. Beyond the scope and volume of information generated, concerted efforts are being made to integrate the information into a better understanding of the relationships across all levels of the taxonomic hierarchy from species to kingdoms. The Angiosperm Phylogeny website (APweb; Stevens 2001–) is particularly notable for the breadth and depth of information it includes and because it is regularly updated. Although nominally focused on flowering plants, APweb also includes information on the seed plants as a whole and on their sister group, the ferns sensu lato. Ex situ samples need not be limited to seed plants. Other plant groups appear to be amenable to long-term storage, including ferns and so-called fern allies, such as *Equisetum* (Ballesteros et al. 2012) and even bryophytes (Pence 2008, 2014). Soil and other symbiotic microflora are important and largely neglected in the context of banking seeds (Merritt et al. 2014).

Phylogenetic information, in the form of hypothesized patterns of evolutionary relatedness among organisms, can be used to develop an "expected" distribution at any level of the hierarchy, against which an empirical distribution of sampled or proposed taxa can be evaluated. Nevertheless, even with perfect knowledge of the relationships among taxa, there is no single correct way to choose among taxa for conservation purposes. The challenge is complicated by the fact that for any
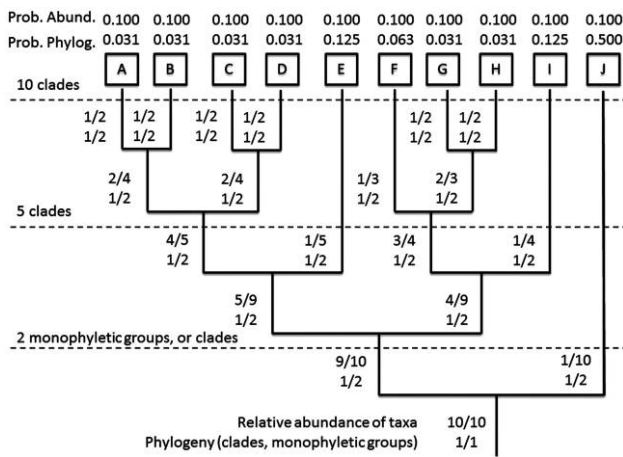
Fig. 2 A hypothetical phylogenetic tree showing relationships of 10 terminal taxa, A–J, to illustrate some approaches for sampling monophyletic groups. The group reflects nine cladogenic (or lineage-splitting) events by which a single common ancestor gave rise to 10 terminal taxa. After the first cladogenic event, there are two monophyletic groups; one comprises nine terminal taxa (A–I), the other a single terminal taxon (J). The pattern continues until there are only terminal taxa. The lower of the two fractions at the base of each monophyletic group—in this case, always 1/2—represents the two products of a lineage-splitting event. If there had been any trichotomies, each product would have had the value 1/3. The upper value of the two fractions represents the relative abundance of terminal taxa resulting from each lineage-splitting event. The two rows of probabilities along the top are the product of all diversification values leading to each terminal taxon; the upper represents probabilities based on abundance, the lower those based on phylogenetic history. The three horizontal dashed lines indicate the situation with two, five, and 10 monophyletic groups, the summary values of which are depicted in figure 3.

given sample size, the combinations of terminal taxa that constitute a representative sample at one level in the hierarchy might not be representative at one or more other levels. So, too, there is no single criterion for choosing particular taxa for conservation, and different purposes may result in different suites of species being chosen.

The earliest attempts to use phylogenetic information to advance conservation were focused on identifying natural area reserve sizes and locations to maximize phylogenetic diversity (Erwin 1991; Vane-Wright et al. 1991; Faith 1992). A recent review by Winter et al. (2013) states that "there is little evidence that phylogenetic diversity has contributed to nature conservation" (p. 199). They attribute this in part to a bewildering array of metrics and indices that have been proposed. We seek here to raise the issue of phylogenetic representation in a simpler manner to illustrate some of the basic issues as they concern species selection for ex situ plant conservation.

To ground the discussion of selecting taxa for collection with respect to their phylogenetic relationships in a concrete example, consider a monophyletic group of 10 terminal taxa named A through J, the phylogenetic relationships among which are illustrated in figure 2. Two of presumably many possible approaches to target taxa for collection are explored

here: one holds that any and all products of a cladogenic event have equal standing, and the other emphasizes the relative numbers of terminal taxa in the products of a particular cladogenic event (fig. 3). In effect, the former emphasizes the phylogenetic isolation or distinctiveness of particular taxa—that is, those with the deepest phylogenetic roots—and the latter favors more speciose groups, which presumably have demonstrated an ability to diversify. These can be thought of loosely as the "branch" or "twig" strategies, respectively.

If all terminal taxa are considered to be independent of one another statistically, then a collection of x taxa could, in effect, serve as the "observed" to be compared with the "expected" (each having an expected value of 0.1) using a χ^2 test. If rarity, for example, was the primary criterion, then species-rich genera (such as *Astragalus* in North America) that have many rare taxa would tend to be emphasized. However, terminal taxa (species, subspecies, varieties, ecotypes, etc.)—the entities with which we have to work—are not independent of one another. Rather, they are the product of evolutionary history, such that to select any terminal taxon is also to select a series of ever more inclusive monophyletic groups. A rigorous statistical analysis evaluating a seed bank's holdings relative to the appropriate sampling universe viewed phylogenetically (and simultaneously across different levels of the hierarchy) is beyond
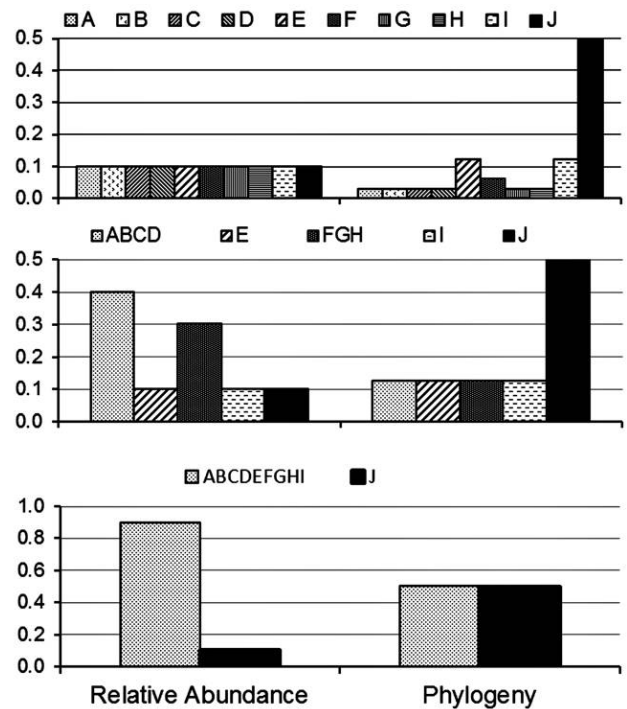
Fig. 3 Histograms graphically showing the expected values based on relative abundance and phylogenetic history for two, five, and 10 monophyletic groups. Note how the disparities among taxa of two approaches are roughly opposite one another, with the disparity among taxa decreasing as numbers of taxa increase, as judged by relative abundance of terminal taxa, and increasing as numbers of taxa increase, as judged by phylogenetic history. The probabilities indicated in the two rows above the phylogenetic tree in figure 2 are depicted graphically in the upper histogram showing 10 terminal taxa.

the scope of this treatment. Our purpose here is to raise the question of how to best sample an array of taxa within the context of their phylogenetic relationships and to offer the beginnings of a comprehensive approach: how are we to use phylogenetic information in choosing taxa for ex situ conservation?

The utility of this approach is that it provides a way to generate expected values against which each and every monophyletic group can be compared with the observed values of taxa in a seed bank. The expected values can be based on different criteria, each presumably emphasizing some particular point of view (figs. 2, 3). The phylogenetic method emphasizes phylogenetic distinctiveness, favoring long-lived groups that have not diversified. Alternatively, using relative abundance in a phylogenetic context will emphasize the products of cladogenic events that have the property of having diversified relatively recently. Neither criterion is inherently superior to the other, and each emphasizes a property worthy of conservation. Ultimately, some combination of both branch and twig strategies may provide the best path forward. For example, one simple way would be to use the mean values of each group at each level of the hierarchy as the expected value against which to compare taxa in a collection. In that way, both extremes are recognized without unduly depreciating more central values in both measures. Given the long-term nature of sampling a flora for ex situ conservation, these sorts of analyses will give seed banks an explicit methodology for evaluating the holdings with respect to their phylogenetic relationships and help inform future sampling choices.

Environmental and Ecological Factors

Species distributions are the product of a great many historical, environmental, and ecological factors, so it is useful to subdivide a seed bank's focal area into smaller, relatively homogeneous geographic units from which representative species can be chosen. Some species will be limited to a single ecogeographic unit, and other, more widespread species will occupy multiple units. It is important to recognize and to collect both sorts of species.

There are many ways large areas can be subdivided into biologically meaningful geographic units, and these can be used to stratify sampling across the landscape. The concept of ecoregions is gaining popularity as a method of identifying biologically meaningful subdivisions within a larger landscape. The World Wildlife Fund developed a global system of more than 800 ecoregions (http://www.worldwildlife.org/biomes), which it defines as a "large unit of land or water containing a geographically distinct assemblage of species, natural communities, and environmental conditions." They are too coarse to be of much utility for many parts of the world, such as Taiwan, which in this system has only two ecoregions. However, in the United States, the Environmental Protection Agency (EPA) has organized a major effort to delineate ecoregions in North America (http://www.epa.gov/wed/pages/ecoregions.htm), on the basis of Omernik (1987). Ecoregions are based on a combination of biotic and abiotic phenomena, including geology, physical geography (elevation, slope, aspect, etc.), vegetation, climate, soils, land use, wildlife and hydrology, which together reflect ecosystem quality. The EPA ecoregions are defined for four hierarchical levels, with level 1 comprising the largest, most inclusive units and level 4 the smallest, least inclusive units. These have proved useful in North America as a way to divide large areas into more manageable and biologically meaningful units that are reasonably homogeneous within themselves and distinct from others. Their utility with respect to choosing species for collection may help ensure that all major environmental features are taken into consideration. Given that the ecoregions reflect some of the same objectives pursued in a seed banking mission, the use of these boundaries may be particularly useful for defining a collection strategy that reflects environmental patterns driving both among- and within-species diversification.

Where ecological restoration is a primary purpose, a well-defined restoration strategy can be used to inform species selection. For example, the US Forest Service and Bureau of Land Management control vast tracts of land, especially in western states, where much of the restoration efforts are focused on range land or steppe vegetation. Much of their collection focuses on "workhorse species," which they characterize generally as local native taxa that have broad ecological amplitudes, are easily collected and propagated, and grow well on disturbed sites (http://www.fs.fed.us/wildflowers/nativeplantmaterials/speciesselection.shtml). These criteria are intended to identify plants that are suitable for postfire rehabilitation and restoration, stabilization of disturbed riverbanks and stream banks, and other situations where there is a need to establish a suitable cover of native vegetation to resist invasive species and that support longer-term recovery.

Another excellent example of a clearly articulated restoration strategy informing the choice of species selection—in this case, tropical forests—can be found in *How to Plant a Forest: The Principles and Practice of Restoring Tropical Forests*, in which scientists at Thailand's Chiang Mai University's Forest Restoration Research Unit developed the concept of "framework" species (Forest Restoration Research Unit 2005; http://www.forru.org/en/content.php?mid=87). The concept was conceived in Queensland, Australia (Goosem and Tucker 1995), for restoration of tropical wet forests and has been applied successfully for recovery of seasonally dry tropical forests in Thailand. In this book-length treatment, the authors develop the concept of accelerated natural regeneration. The key ecological characteristics of framework tree species are not unlike those of workhorse species. They are indigenous species that have high survival rates when planted out in deforested sites; that exhibit rapid growth with dense, spreading crowns that shade out herbaceous weeds; and that flower and fruit at a relatively young age such that they attract seed-dispersing wildlife. The authors advocate a mix of early and later successional species, in part to accelerate natural regeneration.

How Many and Which Populations to Sample?

Once taxonomic priorities are established, establishing sites or populations to target for actual collection is often more complex. Collecting from between 5 and 50 populations is generally recommended depending on the planned uses of the collections (see Brown and Marshall 1995; Guerrant et al. 2004a). Sampling should be stratified over the entire range of the species. Neither taxa nor environmental characteristics of habitat are randomly distributed across the landscape, and

both have bearing on sampling choices. To ensure target taxa are sampled in the most representative way practical and given the enormity of the task, it may be desirable to stratify the sampling effort by one or more geographic or environmental factors.

Stratification refers to the process of dividing a group, such as a species' range, into two or more subgroups with respect to some feature of the geography or environment. For example, a target taxon might be found across an elevational range of 500 m, and the strata could arbitrarily be defined in 100-m intervals, such that the entire sample for the taxon includes members from across the species' elevational range. Not all strata need to have a similar number of samples taken (Elzinga et al. 1998), and actual sampling design can legitimately weight each stratum by some stated reason, say, the relative abundance of that species in that elevational band.

Geographic Factors

It is commonly assumed that species are most abundant in the central portion of their ranges. Some might then direct collections toward range edges by asserting that edge populations are genetically distinct but harbor less variation and are more prone to extinction than those in the center (Sagarin et al. 2006). Specifically, edge populations are assumed to be isolated and to experience reduced reproductive output, thus decreasing within-population genetic diversity and increasing among-population differentiation. However, this is not well supported empirically, with fewer than 40% of studies finding general support for the idea (Dixon et al. 2013; reviewed in Sagarin et al. 2006). A meta-analysis of such studies showed low neutral marker variation between central and peripheral populations across both plant and animal species but also supported the general trend that there is higher marker variation in the center of a species range, which declines toward the periphery, and more differentiation among peripheral populations (Eckert et al. 2008). However, peripheral populations (geographically marginal), especially those at the limits of their range (ecologically marginal), may possess unique solutions to evolutionary pressures (Lesica and Allendorf 1995). For example, Darling et al. (2008) found that traits associated with increased seed dispersal are found more frequently at range edges.

Therefore, creating a collection strategy that simply concentrates efforts in the either the central or the peripheral portion of a species' range is not likely to capture the broadest array of genetic diversity and, more importantly, may not capture the array of ecological or local adaptation represented across the range of a species. A broad representative distribution of collection sites across a species' entire range and stratified across environmental features such as soil or climate should better capture adaptive variation.

Environmental Factors

Within a region, there are likely to be geographically restricted habitats that harbor populations with unique adaptations. For example, in the Midwestern United States, dolomite prairie is rare and gives rise to a distinct subset of species that are more or less restricted. Other more common and widespread species, however, are likely to be ecotypically distinct when found in dolomite-dominated sites and might therefore be targeted for collection if genetic distinctiveness is a priority. Other examples include edaphic or soil endemics or species that are found only with a narrow elevational band. Taxa that have narrow range limits or restricted distributions may be desirable to target. Any biodiversity hotspots within the geographic region are likely to be considered high-priority areas to target for seed collection. Such hotspots could be identified by classic gap analysis and may already be well known. The use of ecoregional boundaries, which summarize relevant biotic and abiotic information across vast landscapes, to create and prioritize a seed collection strategy within taxa is a way to combine both environmental and geographic approaches.

Seed Transfer Zones

Just as species are not uniformly distributed across the landscape, not all populations of a species will necessarily be well adapted to other parts of the species' range—indeed, they can be significantly maladapted. The classic reciprocal transplant common-garden studies by Clausen et al. (1940, 1948) dramatically demonstrated this as early as the 1940s. This means seed sources used in restoration efforts need to be matched to geographic locations for which they are suited. This phenomenon led to the development of the concept of seed transfer zones, which originated with the forestry community in the 1950s; these are geographically bounded and ecologically similar regions where seed can be collected and used for restoration with a higher rate of success. The US Forest Service noticed that most nonlocal plantings of Douglas fir (*Pseudotsuga menziesii*) trees in Oregon did not survive a prolonged cold spell, but local trees suffered little damage (Johnson et al. 2004). Since that time, the US Forest Service has delineated seed transfer zones for numerous timber species on the basis of their patterns of adaptive genetic variation within and among populations. However, work on grasses and forbs has been more recent and relatively limited. It is also important to note that given our changing climate, seed zones must be dynamic (or "floating"), incorporating geography, geology, and shifting climates (Billington and Pelham 1991; Rehfeldt 2004; Ying and Yanchuk 2006; Kramer and Havens 2009).

Seed zone delineation is a costly and time-consuming process involving large-scale common-garden experiments done on a species-by-species basis. Since the vast majority of plant species have not yet been assessed, some recent US Forest Service research efforts have focused on the development of provisional seed transfer zones. These zones started with geographic information system maps of climate variables (winter minimum temperature [December–February], maximum mean monthly temperature, and annual precipitation) that were overlaid with Omernik (1987) level 3 ecoregions. The authors propose using zones based on minimum winter temperature and annual precipitation for woody species and zones based on average maximum temperature and annual precipitation for grasses and herbaceous plants (Bower et al. 2010). They emphasize that these provisional zones are a starting point that should be adjusted on the basis of information about the species, its genetic patterns, and the microsites where it grows, as available. For instance, species that are highly outcrossing and have

large neighborhood sizes are likely to have larger seed transfer zones than those that are highly selfing with small neighborhood sizes. In the absence of information on appropriate seed transfer zones for particular taxa, the option of selecting different sources for specific restoration efforts can be left open by keeping all samples separate and preferentially using the closest or most ecologically similar sources when the need arises.

How Many and Which Individuals to Sample?

Questions of sampling intensity and order are unending and are as relevant to newly established seed banks as they are to more mature programs. For a variety of reasons, target numbers originally provided by the Center for Plant Conservation (1991) for conservation collections of rare plants have been revised significantly upward (Brown and Marshall 1995; Guerrant et al. 2004*a*; Seeds of Success 2012). In general, large seed lots collected from at least 50 individuals within each population are desirable. Individuals should be selected in a stratified random fashion so that plants from different microsites within the population contribute to the seed collection.

Single or Multiple Collections?

Seed banking programs generally try to maximize the genetic diversity of the seed collections while minimizing the cost and effort to collect. The decision about whether to collect from a site once or multiple times can be influenced by several factors. First, is it possible to get an adequate collection in one visit without harming the population? If not, multiple visits should be considered. Guidelines regarding safe levels of harvesting have been developed by Menges et al. (2004). Second, spreading collections over several seasons will likely capture more genetic diversity. Each year weather conditions will favor some genotypes over others, and by collecting over many years you will be more likely to capture seeds from plants adapted to various conditions. Similarly, plants bloom and ripen seed temporally across a season. Multiple collections, either within a season or between seasons, will capture early-, mid-, and late-blooming plants. Spreading collection efforts temporally may be as important as spreading them spatially in terms of capturing genetic diversity. For example, in *Echinacea angustifolia*, the timing of seed harvest affects both seed quantity and quality. Early-fruiting plants had significantly fewer and different pollen donors than later-fruiting plants. Late-fruiting plants matured fewer seeds with lower germination percentages than plants harvested at the peak of seed maturation (Ison 2010).

The question of single or multiple collections applies also to populations. Under what circumstances is it appropriate to collect from only a single population, and when is it preferable to collect from multiple populations across a species' range? Ultimately, the answer depends on the purpose of the collection.

Maternal Line or Bulk Collections?

The decision about whether to collect and accession material by maternal lines (seeds from each individual plant kept sep-

arately) or in bulk (all seeds from a population on a particular day put into a single collection) again rests in part on the purpose of the collection. Maternal line collections offer much more flexibility in the future for reintroduction or research projects where equalizing contributions from each family or maternal line is desired. Equalizing family size in a reintroduction maximizes the effective population size and may help prevent genetic problems caused by genetic drift and artificial selection (Havens et al. 2004). This may be particularly important in rare-plant reintroductions. However, because of the extra time and expense associated with maternal line collections, seed banks typically make bulk collections, especially for more common taxa and for those that could be easily recollected in the future if maternal line collections become necessary.

Synthesis: A Case Study

As we discussed earlier, choosing taxa to target for collection depends largely on the purposes and goals of the collection. Even then, it can be difficult to determine which species should be targeted and prioritized for collection. The DNTPSB at Chicago Botanic Garden chose to prioritize collection of taxa used for restoration in the Midwestern United States. Toward that end, a list of all seed-bearing plant species that occur in 12 ecoregions (EPA level 3) representing the core of the tallgrass prairie region (fig. 1) was compiled using the NatureServe database (http://www.tinyurl.com/bq75pop), eliminating nonnative taxa and those of recent hybrid origin. Also eliminated were taxa that produce recalcitrant seeds, resulting in a preliminary target list of 3000 species. This resulted in the list of species targeted for a single representative sample of the species (Vitt et al. 2010).

A prioritization process was used to narrow the list to ~580 species important for restoration. For these taxa, multiple collections across the species range are made, attempting to sample at least one population from each of the ecoregions where it occurs. Regional floras, vegetation surveys, and other published accounts of the regional and local floras were used to determine the importance or dominance of a plant species in common habitats (prairie, woodland, savanna, marsh, etc.). Geographic range maps were used to confirm the primary distribution of a species, and other information on the ease of establishment or overall performance in habitat restoration projects was also used to determine which species to include. Each species was evaluated in each of the ecoregions in which it occurs and assigned a numerical rank:

1. A conservative species that is considered important to the most common communities (prairie, savanna, marsh) in the ecoregion. "Important" is defined as being dominant in or characteristic of a community, and "conservative" means that the species has high fidelity to relatively unaltered high-quality natural areas.

2. A conservative species that is considered less important to a common community (prairie, savanna, marsh) and less conservative species that are often used in seed mixes for habitat restoration.

3. Less conservative species that are important to a common community (prairie, savanna, marsh) and species that are occasionally used in seed mixes for habitat restoration.

Species that were categorized as either 1 or 2 in at least one ecoregion became the target list of 550 restoration taxa. In consultation with the restoration community or local seed collectors, species are added or subtracted from the list. Seed bank staff further ranked the species to determine their collection priority. As each species has a rank in each ecoregion, the following criteria were used to determine our collection priorities: (1) all species designated 1 in at least five of the ecoregions in which they occur, (2) all species designated 2 in at least five ecoregions, or (3) all species with at least one designation of 1 and four other number designations in at least five ecoregions. This resulted in 171 top-priority species.

In the end, the DNTPSB developed a strategy to bank virtually the entire native seed-bearing flora of the upper Midwestern United States but at different levels of intensity. At the broadest level, 3000 species will have a bulk collection from at least one population. Nearly 600 species important to restoration efforts will be sampled across their range, with at least one population collected from each of 12 level 3 ecoregions. The restoration collections are also bulk collections. Last, for the regions' ~25 globally rare species, maternal line collections are banked from as many populations as are possible and prudent to sample.

Conclusions

Given the ever-increasing threats facing plant species in the wild and the currently limited capacity for restoration, the need for seed banking is acute and growing. Seed banking is both technologically feasible and relatively inexpensive for the vast majority of plant species. With climate change and an uncertain future for natural ecosystems, collecting and banking seeds from as many plant species as possible makes good sense. Similarly, striving for large and genetically diverse seed collections is desirable, as long as care is taken not to further endanger wild populations. In the end, seed collection activities will probably always be a balance of the strategic and the opportunistic, but taking the time to thoughtfully plan and prioritize collections can make the most of limited resources. We must act quickly and collaboratively to ensure that we conserve as much plant diversity as possible for future generations.

Acknowledgments

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