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INVITED PAPER

For the Special Issue: Evolutionary Insights from Studies of Geographic Variation

Project Baseline: An unprecedented resource to study plant evolution across space and time[1]

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PREMISE OF THE STUDY: Project Baseline is a seed bank that offers an unprecedented opportunity to examine spatial and temporal dimensions of microevolution during an era of rapid environmental change. Over the upcoming 50 years, biologists will withdraw genetically representative samples of past populations from this time capsule of seeds and grow them contemporaneously with modern samples to detect any phenotypic and molecular evolution that has occurred during the intervening time.

METHODS: We carefully developed this living genome bank using protocols to enhance its experimental value by collecting from multiple populations and species across a broad geographical range in sites that are likely to be preserved into the future. Seeds are accessioned with site and population data and are stored by maternal line under conditions that maximize seed longevity. This open-access resource will be available to researchers at regular intervals to evaluate contemporary evolution.

KEY RESULTS: To date, the Project Baseline collection includes 100–200 maternal lines of each of 61 species collected from over 831 populations on sites that are likely to be preserved into the future across the United States (~78,000 maternal lines). Our strategically designed collection circumvents some problems that can cloud the results of "resurrection" studies involving naturally preserved or existing seed collections that are available fortuitously.

CONCLUSIONS: The resurrection approach can be coupled with long-established and newer techniques over the next five decades to elucidate genetic change and thereby vastly improve our understanding of temporal and spatial changes in phenotype and the evolutionary processes underlying it.

KEY WORDS climate change; geographic variation; natural selection; phenotypic evolution; population differentiation; resurrection ecology; seed bank; species range

The process of evolution is central to biology, and understanding how it operates in natural populations continues to be a major goal. Originally thought to proceed extremely slowly (Darwin, 1859), evolutionary changes occurring over the scale of a century were identified considerably later (e.g., Antonovics and Bradshaw, 1970), and there is now abundant evidence that contemporary evolution is often much more rapid than previously assumed (reviewed by Koch et al., 2014). Anthropogenic disturbance to natural habitats, including climate change, continues to be a potent driver of evolution. Because selection varies temporally and spatially and populations have unique histories and genetic compositions, the rate and extent of evolutionary response are expected to vary across species' ranges. Thus, anthropogenic disturbance offers opportunities for studies of diverse evolutionary responses in wild species across both time and space.

Evolutionary change can be studied directly using both temporal and spatial comparisons (Antonovics, 1976; Franks et al., 2014). Despite these recognized approaches, investigation of evolution jointly over both large spatial extent and decadal temporal scales is hindered by inaccessibility of suitable biological material. This

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paper describes a new seed collection, Project Baseline, which is specifically designed to provide a temporal sample of seeds collected in 2013–2015 for future investigation of evolutionary dynamics of traits across plant species' geographic ranges in the contiguous United States. The fundamental idea is that over the next 50 years, researchers can withdraw seeds from this living genome bank, recollect seeds from conspecific populations at the same and other locations, and compare the distributions of traits of plants from antecedent and successor populations by growing them in a common environment, a method commonly known as the "resurrection" approach (Hairston et al., 1999; Franks et al., 2008).

The Project Baseline collection is extensive, encompassing diverse species and numerous populations of each, with genetic structure retained for each population sample. This sampling scheme is designed to capture both genetic variation within populations and divergence between populations. Changes at both levels of diversity are expected as populations continually undergo all the evolutionary and demographic processes that can collectively result in range expansion, adaptation, or extinction (Davis et al., 2005). With this valuable resource, evolutionary biologists can conduct resurrection experiments with plants sampled from the same locations decades from now and generate evolutionary insights using long-established approaches and/or newer ones as well as others that are yet to be developed.

Our purpose here is to raise awareness about this unique open-access resource available to the scientific community. To this end, we first provide an overview of spatial and temporal approaches to studying contemporary evolution. Second, we describe the Project Baseline goals highlighting aspects of the sampling design that are particularly valuable or unique. Third, we detail the species and populations in the collection. Fourth, we describe some of the pitfalls of resurrection experiments that can be minimized using seeds in this collection. Finally, we describe how researchers can obtain access to the collection and conclude with a description of how Project Baseline vastly expands opportunities to study evolution in natural and naturalized plant populations over the next half century.

Spatial and temporal approaches to understanding evolutionary change—In the use of a space-for-time substitution to study contemporary evolution, genetically based divergence in phenotypic or genetic attributes among spatially separated populations is generally taken to reflect evolutionary changes over time. For example, divergence in allele frequency or phenotypic traits across an environmental gradient suggests evolutionary change from a common ancestral population. Similar patterns of population differentiation across parallel clines in different locations provide further support of such change and indicate repeated evolutionary divergence (Hoffmann and Weeks, 2007; Colautti and Barrett, 2013). If evidence of phenotypic divergence is coupled with reciprocal transplant experiments that show home-site advantage (i.e., local adaptation), then it can be inferred that populations have diverged through response to natural selection under differing environmental conditions (e.g., Etterson, 2004). A substantial body of such evidence for local adaptation exists for plants (Leimu and Fischer, 2008).

In contrast, the resurrection approach can be used to directly study contemporary evolution over time (Bennington et al., 1991; McGraw et al., 1991; McGraw, 1993; Hairston et al., 1999). Here "resurrection" refers to raising organisms that have persisted in dormancy as propagules such as seeds or dormant eggs. When ancestral and contemporary propagules are raised in common conditions, differences between the phenotypes and their underlying genetics reflect the nature of evolutionary change that has occurred during the intervening time interval. Demonstrating phenotypic differences alone between populations observed in situ at different times is not sufficient to demonstrate evolution, since phenotypic differences can also arise due to plasticity (Allard and Bradshaw, 1964; Etterson, 2004; Merilä and Hendry, 2014). For example, widespread shifts toward earlier initiation of flowering in many plant populations have been detected in conjunction with progressive global warming, contributing to an increase in the length of the flowering season in regions where flowering is temperature-dependent (Fitter and Fitter, 2002; Parmesan and Yohe, 2003; Miller-Rushing and Primack, 2008). Without additional information, it is not possible to determine whether these shifts are due to plasticity, evolutionary responses to selection for earlier flowering, or both.

The resurrection approach can empirically distinguish evolution from plasticity. This approach has been used with preserved propagules harvested from nature (e.g., McGraw et al., 1991; Hairston et al., 1999), seeds fortuitously available in storage (e.g., Franks et al., 2007; Nevo et al., 2012; Sultan et al., 2013; Thomann et al., 2015), and in the laboratory in conjunction with experimental evolution studies (e.g., Lenski et al., 1991; Fox and Lenski, 2015) and mutation–accumulation studies (e.g., Schultz et al., 1999; Shaw et al., 2000). These studies have demonstrated evolution over substantial timescales and have underscored that evolutionary change in a variety of morphological and life history traits can be an important component of biotic responses to shifts in environmental conditions. Thus far, most resurrection ecology experiments of wild populations have relied on propagules available through serendipitous opportunity. However, genetic material in the form of seeds can be strategically collected and preserved in a way that facilitates resurrection experiments and enhances the quality of inferences from this work.

Goals of the Project Baseline initiative—The word "baseline" in the collection's name reflects our objective to provide a reference point for population-level phenotypic and genetic attributes with which future populations can be compared following evolutionary responses to environmental change. As such, this collection differs from other seed banking efforts in both its scope and its goal of preserving genetic resources specifically for research. Valuable seed banks have been established with the primary goal of conservation (e.g., The Millennium Seed Bank; Royal Botanic Gardens, 2014), restoration of native plant communities on a landscape level (e.g., Seeds of Success; Bureau of Land Management, 2014), or preservation of agricultural resources as a basis for plant breeding (e.g., National Center for Genetic Resources Preservation; USDA, 2015). Project Baseline includes fewer species than these collections, but each is represented, both genetically and spatially, by seeds collected from 100 to 200 individual maternal lines within numerous populations spanning environmental gradients across each species' geographic range. Many attributes of the collection described below were designed in consultation with 30 plant evolutionary biologists and seed storage experts at a workshop held before project initiation (Franks et al., 2008).

Species in the collection represent a diversity of life-history characteristics, growth forms, functional groups, range sizes, and

native/nonnative status. We have included species that are of present interest to the scientific community based on published studies and some that are currently understudied. Two aspects of our sampling design facilitate resurrection experiments. First, seeds have been sampled from populations at locations that are likely to remain protected in the upcoming decades (e.g., national parks, state parks, private reserves, biological field stations). By primarily selecting preserved sites, we have increased the likelihood that a population will be available for recollection in the future even though its genetic composition may be altered dramatically. Yet even in cases where a population does not persist at a location where it was originally collected for the Project Baseline, the archived seeds will have value for studies of evolutionary rescue (Gomulkiewicz et al., 2010) by exemplifying instances where evolutionary processes do not suffice to forestall extinction. Second, for each species, populations were sampled according to a standardized protocol with the specific aim of obtaining a representative genetic sample. These protocols are documented such that they can be repeated in the future, which improves the basis of comparison between temporal samples.

To enhance the inferential power of experiments based on this collection, we included one or more congeners, when possible, to permit phylogenetically informed comparisons (Table 1). Sampling multiple populations of closely related taxa across environmental gradients (e.g., multiple species of *Andropogon*, *Bromus*, *Dalea*, *Geranium*, *Helianthus*, *Impatiens*, *Penstemon*, *Rumex*, and *Triodanis*) can serve as a basis for identifying putative adaptations to contrasting abiotic environments. The repeated phenotypic comparison of congeners occupying contrasting environmental conditions (e.g., dry vs. mesic habitats, soils of high vs. low water-holding capacity, core vs. marginal populations within a species' geographic range, or warm vs. cool temperatures) can enable the identification of adaptations to alternative climatic or microenvironmental regimes (Mazer, 1990; Wang et al., 2014). Moreover, more than one species was frequently collected from the same site, which will permit comparison of evolutionary temporal and spatial responses of different community members and changes in interspecific interactions.

Seeds from every population in the collection are maintained separately by maternal line. This sampling approach demanded considerable labor and time, but it greatly enhances the value of the collection by preserving the genetic structure of populations with a degree of resolution not available with the bulk sampling that characterizes many efforts to preserve the seeds of rare or endangered species. The availability of maternal lines will aid investigators in tracking changes in quantitative genetic parameters (including genetic variance, heritability, and genetic correlation structure) that we may expect to be altered directly and indirectly by climate change and other anthropogenic stressors. For example, investigators will be able to test whether genetic variation has been depleted over time. Changes in standing genetic variation may be expected for many reasons: for example, as a result of drift in small, isolated population remnants; drastic changes in population size, changes in pollinator behavior or abundance and, consequently, plant mating system, temporal changes in patterns of gene flow associated with fragmentation of populations, or changes in the behavior or abundance of seed dispersal agents. Project Baseline will greatly improve our ability to assess how fundamental quantitative and population genetic parameters change in conjunction with contemporary environmental change.

Description of the Project Baseline Collection—As of midsummer 2015, we have collected seeds from 61 species (831 populations) at 166 sites within 39 states of the contiguous United States (~78,000 maternal lines). The collection includes populations representing all major biomes within the sampling area (EPA Level 1 ecoregions) and 64% of more fine-scale ecoregion divisions (EPA Level II ecoregions; U.S. EPA, 2013). Our short-term goal is to complete collections in 2015 such that each species is represented by 20 populations (two populations per site at 10 sites) that span geographic and environmental gradients across the species range (Project Baseline, 2015). A summary of our collection can be monitored in an instantly updated table (http://baselineseedbank.org/collection.php) and collection maps (http://baselineseedbank.org/collection_populations.php). Our longer-term goal is to expand the collection to include accessions sampled from each species' entire geographic range, including (for some species) accessions from Canada, Mexico, and other countries. In addition, we want to augment the collection with accessions of new species and populations, especially from young investigators, who are likely to use this resource in the future.

As an example of Project Baseline's targeted species, our collection includes *Andropogon gerardii* (Poaceae), a native perennial grass that is common in tallgrass prairie ecosystems, has a large geographic range, and has been the subject of previous ecological and evolutionary research (e.g., Keeler, 1990; Norrmann et al., 1997) (Fig. 1A). This species is complemented with collections from two congeners (*A. virginicus* and *A. ternarius*) that have more restricted southern distributions and have received scant empirical attention in the literature. Another set of congeners, *Ratibida pinnata* and *R. columnifera* (Asteraceae), are both native forbs of the tallgrass prairie, but *R. columnifera* has a more extensive and western range than *R. pinnata* (Fig. 1B). These species have been previously studied primarily in a community context (e.g., Foster et al., 2002). Populations are now archived from many of the same sites as the dominant prairie species *A. gerardii*. The Project Baseline collection also includes introduced species such as *Melilotus officinalis* (Fabaceae), a ruderal annual legume (Fig. 1C) that has yellow and white flower morphs that are sometimes considered distinct species (i.e., *M. alba*, Wolf et al., 2004). *Melilotus officinalis* is also of interest because populations of this species can differ in lifespan, including annual, biennial, and perennial life histories (Smith, 1927), thereby providing material for testing evolutionary change in lifespan and the number of reproductive events (e.g., Reinartz, 1984; Lacey, 1988). Overall, Project Baseline contains a diverse set of species that will likely continue to expand as researchers submit seeds of their own study species that have been collected according to the project protocols.

Accession, population, community, and site data are archived with the seeds and available for public use via a MySQL relational database that is currently held on a University of Minnesota Duluth server. Supplemental data include a list of common co-occurring plant species at each collection location, estimates of fruiting phenology and percentage cover, slope, aspect, notes about land ownership and disturbance, estimates of population size, photos of the collection location, and voucher specimens. Researchers can query the database or export data on seed collections and associated information through a web interface (http://baselineseedbank.org/search.php). All maps of the web page allow researchers to view collection locations with temperature, precipitation, and ecoregion layers. Voucher specimens are publicly available and are housed at

TABLE 1. A list of species collected for Project Baseline's seed bank thus far, with summaries of ecological roles, life histories, and growth habits.

Species	Family	Native/Introduced	Annual/Perennial	Growth habit
Ambrosia artemisiifolia	Asteraceae	Native	Annual	Forb/herb
Amorpha canescens	Fabaceae	Native	Perennial	Shrub, Subshrub
Andropogon gerardii	Poaceae	Native	Perennial	Graminoid
Andropogon ternarius	Poaceae	Native	Perennial	Graminoid
Andropogon virginicus	Poaceae	Native	Perennial	Graminoid
Apocynum androsaemifolium	Apocynaceae	Native	Perennial	Forb/herb
Apocynum cannabinum	Apocynaceae	Native	Perennial	Forb/herb
Asclepias syriaca	Asclepiadaceae	Native	Perennial	Forb/herb
Aquilegia coerulea	Ranunculaceae	Native	Perennial	Forb/herb
Bouteloua curtipendula	Poaceae	Native	Perennial	Graminoid
Brassica nigra	Brassicaceae	Introduced	Annual	Forb/herb
Brassica rapa	Brassicaceae	Introduced	Annual, Biennial	Forb/herb
Bromus diandrus	Poaceae	Introduced	Annual, Perennial	Graminoid
Bromus inermis	Poaceae	Introduced	Perennial	Graminoid
Bromus tectorum	Poaceae	Introduced	Annual	Graminoid
Chamaecrista fasciculata	Fabaceae	Native	Annual	Forb/herb
Clarkia purpurea	Onagraceae	Native	Annual	Forb/Herb
Clarkia unguiculata	Onagraceae	Native	Annual	Forb/herb
Dactylis glomerata	Poaceae	Introduced	Perennial	Graminoid
Dalea candida	Fabaceae	Native	Perennial	Forb/herb, Subshrub
Dalea multiflora	Fabaceae	Native	Perennial	Forb/herb, Subshrub
Dalea purpurea	Fabaceae	Native	Perennial	Forb/herb, Subshrub
Echinacea angustifolia	Asteraceae	Native	Perennial	Forb/herb
Echinacea pallida	Asteraceae	Native	Perennial	Forb/herb
Echinacea purpurea	Asteraceae	Native	Perennial	Forb/herb
Elymus canadensis	Poaceae	Native	Perennial	Graminoid
Eryngium yuccifolium	Apiaceae	Native	Perennial	Forb/herb
Eschscholzia californica	Papaveraceae	Native	Annual, Perennial	Forb/herb
Euphrasia hudsoniana	Orobanchaceae	Native	Annual	Forb/herb
Geranium carolinianum	Geraniaceae	Native	Annual, Biennial	Forb/herb
Geranium maculatum	Geraniaceae	Native	Perennial	Forb/herb
Helianthus annuus	Asteraceae	Native	Annual	Forb/herb
Helianthus mollis	Asteraceae	Native	Perennial	Forb/herb
Helianthus petiolaris	Asteraceae	Native	Annual	Forb/herb
Hypericum perforatum	Clusiaceae	Introduced	Perennial	Forb/herb
Impatiens capensis	Balsaminaceae	Native	Annual	Forb/herb
Impatiens pallida	Balsaminaceae	Native	Annual	Forb/herb
Larrea tridentata	Zygophyllaceae	Native	Perennial	Shrub
Linum sulcatum	Linaceae	Native	Annual	Forb/herb
Lupinus nanus	Fabaceae	Native	Annual	Forb/herb
Lupinus texensis	Fabaceae	Native	Annual	Forb/herb
Melilotus officinalis	Fabaceae	Introduced	Annual, Biennial, Perennial	Forb/herb
Mimulus guttatus	Phrymaceae	Native	Annual, Perennial	Forb/herb
Panicum virgatum	Poaceae	Native	Perennial	Graminoid
Penstemon australis	Scrophulariaceae	Native	Perennial	Forb/herb
Penstemon digitalis	Scrophulariaceae	Native	Perennial	Forb/herb
Penstemon laevigatus	Scrophulariaceae	Native	Perennial	Forb/herb
Raphanus raphanistrum	Brassicaceae	Introduced	Annual, Biennial	Forb/herb
Raphanus sativus	Brassicaceae	Introduced	Annual, Biennial	Forb/herb
Ratibida columnifera	Asteraceae	Native	Perennial	Forb/herb
Ratibida pinnata	Asteraceae	Native	Perennial	Forb/herb
Rudbeckia hirta	Asteraceae	Native	Annual, Biennial, Perennial	Forb/herb
Rumex crispus	Polygonaceae	Introduced	Perennial	Forb/herb
Rumex hastatulus	Polygonaceae	Native	Perennial	Forb/herb
Salvia lyrata	Lamiaceae	Native	Perennial	Forb/herb
Schizachyrium scoparium	Poaceae	Native	Perennial	Graminoid
Sorghastrum nutans	Poaceae	Native	Perennial	Graminoid
Stipa pulchra	Poaceae	Native	Perennial	Graminoid
Tradescantia ohiensis	Commelinaceae	Native	Perennial	Forb/herb
Triodanis biflora	Campanulaceae	Native	Annual	Forb/herb
Triodanis perfoliata	Campanulaceae	Native	Annual	Forb/herb
Yucca brevifolia	Agavaceae	Native	Perennial	Shrub, Tree

the University of Minnesota Herbarium in the Bell Museum of Natural History in St. Paul, Minnesota and at one of three regional herbaria (Olga Lakela Herbarium of University of Minnesota Duluth, Steere Herbarium of New York Botanical Garden, or Cheadle Center for Biodiversity and Ecological Restoration Herbarium of University of California Santa Barbara). As an aid to further research,

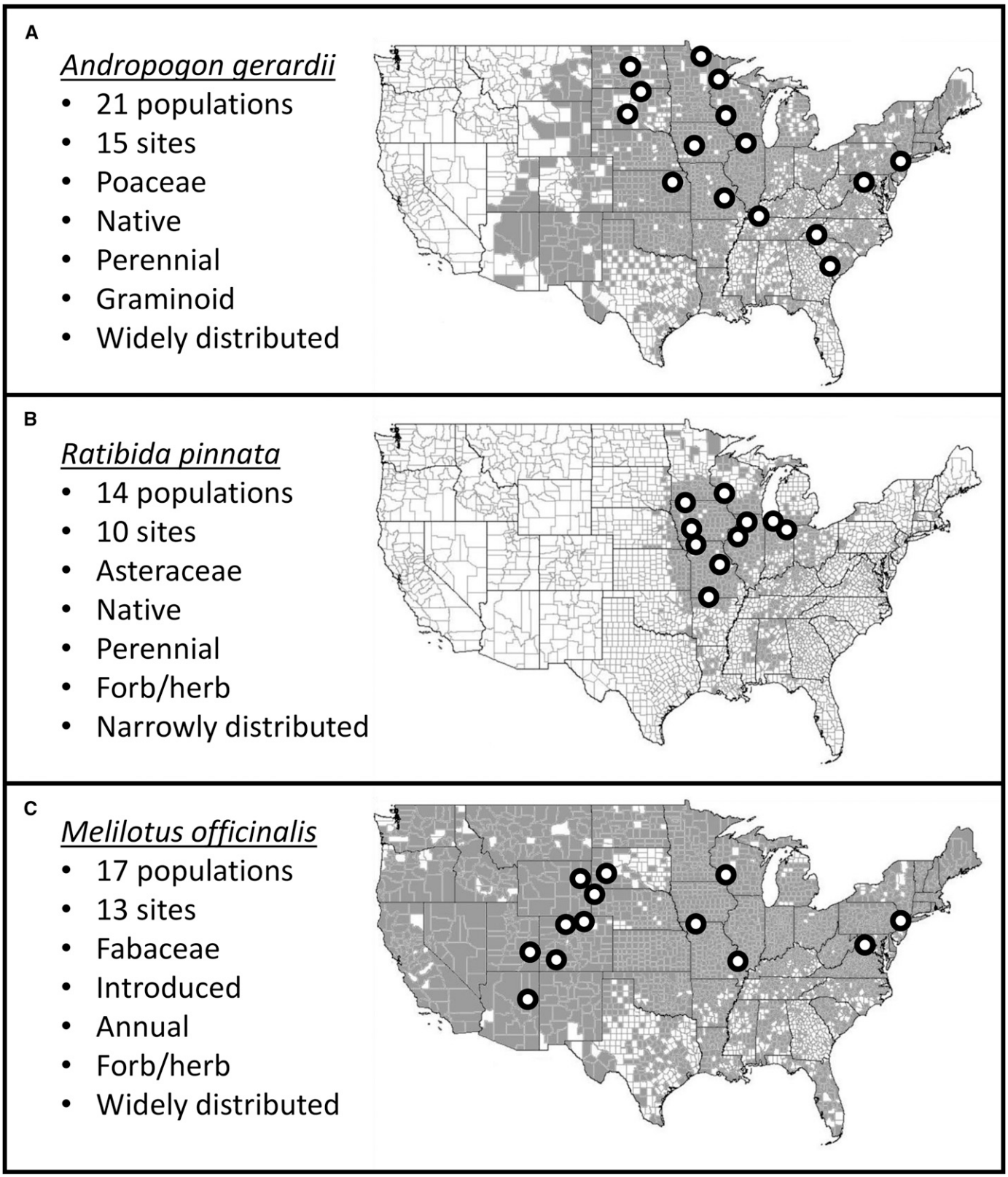

FIGURE 1 Three examples of species in the Project Baseline collection shown with a list of their diverse attributes. Gray designates counties where the species has been documented. Circles mark sites where seeds have been collected for Project Baseline as of 2015.

we have also developed an interactive map that allows researchers to see potential research sites that will be preserved into the foreseeable future and are managed by entities such as the U. S. Forest Service, U. S. National Park Service, U. S. Department of Energy, U. S. National Estuarine Research System, National Ecological Observatory Network, The Nature Conservancy, Long Term Ecological Research Sites, and the Organization of Biological Field Stations (http://baselineseedbank.org/pb_allsitesx.html).

Using the Project Baseline Collection—Seeds are stored at the National Center for Genetic Resources and Preservation (NCGRP, USDA, 2015) and will be made available via a proposal submission and review process at 5–10-yr intervals over the next 50 years. To maximize information obtained from this resource, seeds will be distributed for research use incrementally over the next five decades with some samples reserved for the longest time increment. Requests to use Project Baseline seeds will be through a formal research proposal, with the first solicitation in 2018. The first call for proposals will focus on annual species, which will have had more generations to evolve. Previous studies have demonstrated that rapid evolution over this short interval is detectable (Maron et al., 2004; Franks et al., 2007; Sultan et al., 2013).

Proposals will be evaluated by a review panel on the basis of their potential to contribute to a fundamental understanding of evolutionary processes. Because the collection was established to enable resurrection experiments, research conducted with the seeds will be expected to include comparisons of fitness and other traits between plants grown from both Project Baseline seeds and contemporary seed collections from nature. These comparisons are expected to be done via well-designed experiments in which plants of both cohorts are grown contemporaneously in the same environment. In addition, preference will be given to proposals that capitalize on the unique aspects of the collection, such as proposals that target the study of more than one taxon, range-wide population collections of a single taxon, replicate populations sampled from a single site, sister taxa, and/or within-population genetic structure (maternal lines). The proposal evaluation process will be guided by an Advisory Board that will meet and recruit new members at the annual meeting of the Botanical Society of America.

Confronting the caveats about resurrection studies—Experiments based on the comparison of plants raised from past and contemporary seeds, while powerful, raise several inherent caveats. The most obvious of these is that resurrection experiments are rarely possible simply because predecessor seeds are unavailable for experimentation. In nature, the conditions that preserve the long-term viability of seeds or other propagules are uncommon, and such preserved propagules are difficult to find. Project Baseline addresses this issue by providing an open-access resource where seeds are preserved using best practices, assuring that seeds of high viability and known age and collection history are available for future research.

A second caveat about resurrection studies is that the preservation process itself may impose selective pressure on seeds as a consequence of storage conditions or inherent genetic differences in seed lifespan. When dormant seeds from soil or resting eggs from sediments are sampled, not all individuals can be revived. Questions thus arise: Are the successfully revived zygotes a genetically random sample of their generation, or has selection proceeded through the period of dormancy? These are inherent problems because the resurrection approach is predicated on the ability to revive a random sample of the genetic diversity present in populations. To address this issue, our collaborators at the USDA NCGRP are conducting research using seeds from our collections to improve storage practices, minimize loss of genetic variability over time, and enhance germination rates. The process of seed aging and genetic degradation is being studied on seeds of different ages using well-established approaches (i.e., X-ray analysis, lipid crystallization tendency, and structural mechanic analysis: Walters et al., 2005b, 2010), as well as new experimental approaches (RNA degradation) and modeling (Richards et al., 2010). The integrity of the seed archive over time depends upon minimizing natural selection and attendant genetic changes through the period of storage.

The loss of a significant fraction of seeds during storage can cloud the interpretation of resurrection experiments. Suppose, for example, that a plant trait under study (e.g., flowering time or specific leaf area) is genetically correlated with an unmeasured seed trait that influences survival during freezing and revival. Selection on the seed trait during storage would shift the estimated mean of correlated plant traits expressed after revival, thus biasing the "baseline" for estimating evolutionary change. How large is this bias? Consider the following "worst-case" scenario and a more moderate one, which we derived from calculations using the multivariate breeder's equation (Lynch and Walsh, 1998; Hadfield, 2008). Suppose that 50% of stored seeds fail to revive, that all failures are due to a genetically determined seed trait, and that the seed trait is perfectly genetically correlated with a focal plant trait. These conditions can be modeled as truncation selection on the seed trait, so that the intensity of selection is a direct function of the proportion of the zygotes that revive (Crow and Kimura, 1979; Lynch and Walsh, 1998). Under these extreme assumptions, selection on the seed trait shifts the estimated mean of the correlated trait by ~0.8 standard deviations. For a more moderate scenario, assume that although 50% of seed fail, only half of these failures are due to the seed trait, with the rest caused by unrelated factors. Also assume a more moderate genetic correlation of 0.25 between seed and focal traits. Under these conditions, the shift in plant trait mean is only ~0.04 standard deviations. With even lower failure rates and genetic correlations, the bias becomes inconsequential.

The unique structure of the Project Baseline collection allows future researchers to test for potential bias in their baseline estimate of trait means because seeds are collected and stored as maternal families. The correlation between the maternal family mean for the focal trait and the proportion of the seeds in the family to germinate thus approximates the genetic correlation between the focal trait and putative seed traits affecting survival. A difference in trait mean between a revived predecessor population and a successor population can be confidently attributed to evolutionary change when seed germination rate is high and/or the genetic correlation is low. These quantities cannot be estimated from bulk seed collections or for seeds and zygotes recovered from sediments.

Seeds often lose viability over time, even under ideal storage conditions. Ongoing research will allow us to predict the longevity of each accession using newly developed noninvasive assays of seed quality and simulated aging treatments (Walters et al., 2005a, 2010). Loss of viability over time is a sampling phenomenon that can be modeled as drift (random). Our NCGRP collaborators are working to develop a quantitative metric that can be used to account for seed deterioration over time for each accession in the collection. This metric can be applied to the optimization problem of balancing the integrity of the samples ex situ on the one hand and

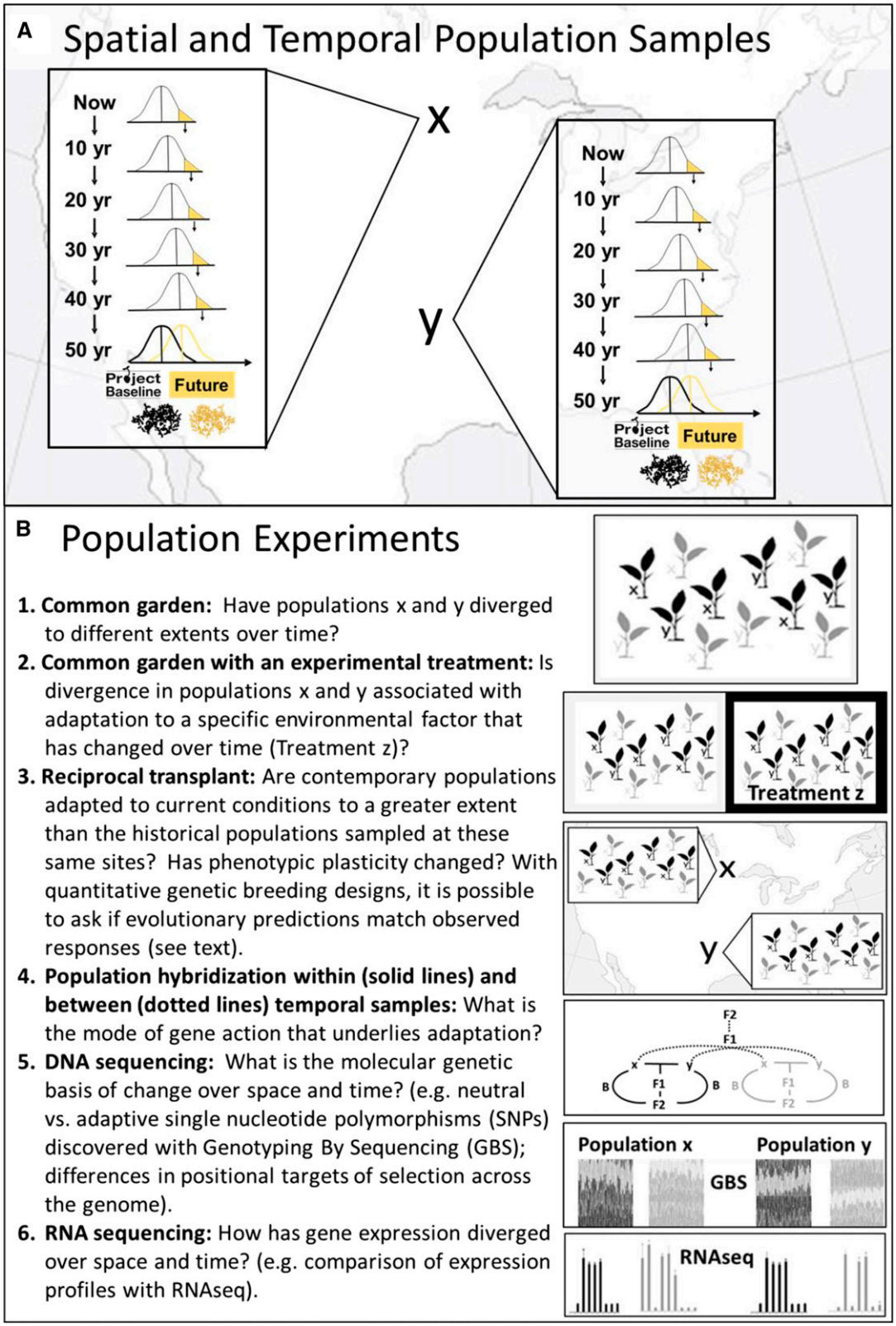

FIGURE 2 Schematic showing hypothetical spatial and temporal population samples as a part of Project Baseline. (A) Two populations, x and y, sampled from different parts of the species range. Seeds collected now as a component of Project Baseline can be compared with seeds collected in the future that have experienced evolutionary processes such as selection (shaded region of distribution), drift, and gene flow over 50 years (change in the mean of the distribution). (B) Antecedent and successor populations can be grown contemporaneously to address an array of evolutionary hypotheses as briefly outlined with six experimental examples. In all panels, Project Baseline and contemporary collections are represented as black and gray, respectively.

the magnitude of environmental change in situ on the other, both of which are time-dependent. Comparisons among wild species and collection sites will offer unique insight into the basis of variation in longevity, especially since there are relatively few seed banks that focus on wild plant species and none that sample as intensively. Ultimately, this work will contribute to the development of better methods for sampling seed diversity and for assessing seed quality.

Project Baseline expands opportunities to study evolution—As a living collection, the Project Baseline seed archive will offer exceptional value as a resource for experiments that directly compare phenotypes of plants from populations sampled at widely different times (and their hybrids) over large geographic areas, but grown contemporaneously in common conditions (Fig. 2A). Innumerable questions can be addressed in this way, and we suggest only a few in Table 2. As a more detailed example, populations within annual herbaceous species or across taxa historically subject to intense late-spring or summer drought may harbor relatively high proportions of genotypes with shorter lifespans, that flower earlier, and have higher selfing rates compared with conspecific or congeneric populations adapted to cooler, moister conditions. Thus, we may predict that populations will evolve more compressed lifespans, earlier flowering, and higher selfing rates in regions where the climate becomes hotter and drier in the future, given suitable genetic variance in these traits. We may further predict that these evolutionary changes result in the maintenance of fitness over time. Such hypotheses can be tested in common gardens (Fig. 2B, Experiment 1) or with experimental treatments (Fig. 2B, Experiment 2).

In addition to straightforward phenotypic comparison between cohorts grown contemporaneously, this seed collection will offer the opportunity for joint quantitative genetic studies of plant populations sampled in the early and mid-21st century using formal genetic crosses within and among populations and generations (Shaw and Etterson, 2012). Exposure of the experimental progeny resulting from intracohort crosses to contrasting environmental conditions (Fig. 2B, Experiment 3) will enable comparisons of the operation of natural selection (i.e., the traits that are subject to selection, of what magnitude and direction) and how this differs with the phenotypic and genetic composition of the cohorts. In cases where estimates of selection are available from prior research on a population in the Baseline collection, it will be possible to judge the precision and accuracy of quantitative genetic predictions of phenotypic evolution (Grant and Grant, 1993). These experiments may indicate limits to the response to selection in nature and shed light on the genetic causes underlying such limits (Etterson and Shaw, 2001). For example, when selection favors trait values that exceed the potential range of expression, whether via response to selection or plasticity, absolute fitness is expected to decline and demographic parameters may fall below the level needed for population replacement, making extinction likely (Chevin and Lande, 2010). The conditions that favor evolutionary rescue from extinction have rarely been studied (Bell and Gonzalez, 2009), especially in wild populations. In addition, crosses between predecessor and successor cohorts would make possible the evaluation of gene action, including epistasis and genotype by environment interaction, contributing to phenotypic evolutionary change (Roff and Emerson, 2006; Franks et al., 2007) (Fig. 2B, Experiment 4).

The Project Baseline seed bank will, in addition, provide opportunities to gain insight into temporal and spatial patterns and processes at the molecular level by applying recent approaches to both antecedent and successor generations. For example, next-generation sequencing technologies are increasingly available at a reasonable cost to researchers who study nonmodel organisms (Fig. 2B, Experiment 5). This trend is likely to continue into the future. Currently, restriction-site associated DNA sequencing (RADseq) and genotyping by sequencing (GBS) can already provide thousands of sequenced markers across many individuals (Davey and Blaxter, 2010; Elshire et al., 2011) that identify single-nucleotide polymorphism (SNPs) and multilocus haplotypes. Progress has been made on distinguishing selected from neutral SNPs (Narum and Hess, 2011). Neutral markers are especially informative for the study of demographic processes, such as gene flow and migration (Beaumont and Balding, 2004). Markers near or within genes under selection provide insight into positional targets of selection across the genome and candidate loci associated with adaptations that have arisen over time (Hohenlohe et al., 2010). Related techniques that sequence the exome (the part of the genome transcribed within mature RNA, i.e., RNA sequencing) can reveal evolutionary divergences in gene expression (Fig. 2B, Experiment 6) (e.g., Schoville et al., 2012). The novelty of questions that can be addressed using quantitative and molecular genetic technique with the Project Baseline source material is only limited by the imagination.

Perhaps the most novel evolutionary insights will be obtained from experiments that merge quantitative

TABLE 2. A short list of targets and agents of selection that will change in the future and corresponding evolutionary predictions that can be tested with the Project Baseline collection.

Targets/Agents	Predictions
Adaptive loci	Selective sweeps occur for loci associated with climate tolerance.
Climate change	Extinction rates increase with the rate of climate change.
Climate variability	Plasticity evolves to a greater extent in variable vs. stable environments.
Dispersal	Wide dispersers diverge more slowly than narrow dispersers.
Drought	Accelerated drought selects for earlier flowering and shorter life cycles.
Elevated CO_2	Selection on water-use efficiency and specific leaf mass declines.
Epistasis	Evolution occurs via fixation of alleles with positive epistasis.
Genetic architecture	Evolution is based on many loci of small effect rather than few loci of large effect.
Genetic constraint	Evolution response is limited by genetic correlations.
Genetic variation	Populations with low genetic diversity have higher extinction rates.
Growing season	Longer growing seasons select for later flowering.
Herbivores	Increased herbivory at northern latitudes selects for defensive traits.
Increased selfing	Purging ultimately reduces the effects of inbreeding depression.
Mating systems	Outcrossing species adapt more rapidly than selfing congeners.
Mutations	Evolution relies on standing genetic variance more than novel mutations.
Ploidy	Diploids evolve more rapidly than polyploids.
Pollination type	Specialist plant–pollinator systems decline more than generalist ones.
Pollinator loss	Floral traits that promote selfing are favored when pollinators decline.
Population size	Drift and inbreeding are stronger in dwindling vs. stable populations.
Rate of evolution	Annuals evolve more rapidly per unit time than perennial congeners.
Selection strength	Genetic variation erodes faster under strong selection.
Temperature	Warming selects for higher optimal photosynthetic temperatures.

and molecular genetic approaches. In particular, when coupled with experimental crosses between plants representing populations sampled at different times and phenotypic evaluation of the progeny, as described already, studies could shed light on the molecular basis of phenotypic change. The number of loci contributing to a specific phenotypic change and the distribution of allelic effects involved persist as engaging questions in evolutionary genetics. The Baseline collection could be used to address this issue for a large set of species subject to coordinated environmental change. We caution, however, that accuracy depends on studies that are extremely large and recombination-rich, as Laurie et al. (2004) demonstrated.

CONCLUSIONS

Given that human population growth, development, deforestation, and climate change are predicted to contribute to the extirpation or extinction of wild plant populations and taxa (Schwartz et al., 2006; Loarie et al., 2008), the opportunities to detect, measure, and understand evolutionary change under natural conditions are dwindling. An understanding of evolutionary responses to anthropogenic influences of both native and invasive species can inform decisions regarding the management, conservation, and restoration of managed lands. In sum, both basic and applied questions regarding the evolution and ecology of wild and naturalized species depend on an understanding of evolution in the context of rapid environmental change. The Project Baseline collection provides an unprecedented opportunity to study these changes over space and time through resurrection experiments, studies of seed longevity, and other research.

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